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FORM**

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July 11, 2003

First Named Inventor

Peter Andersen et al

Art Unit

1614

Examiner Name

Attorney Docket Number

SSI5AUSA

**ENCLOSURES**

(Check all that apply)

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<input type="checkbox"/> Fee Attached	<input type="checkbox"/> Licensing-related Papers	<input type="checkbox"/> Appeal Communication to Board of Appeals and Interferences
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**SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT**

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Signature			
Printed name	Cathy A. Kodroff		
Date	February 15, 2008	Reg. No.	33,980

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SSI5AUSA

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of  
Peter Andersen et al

) Group Art Unit: 1614

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) Examiner:

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Appln. No. 10/617,038

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Filed: July 11, 2003

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For: THERAPEUTIC TB VACCINE

) February 15, 2008

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PO Box 1450  
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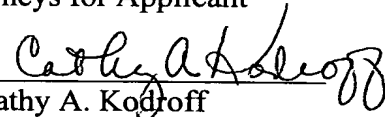
Sirs:

Attached please find the certified copy of the foreign application from  
which priority is claimed in the above-identified U.S. Patent application:

Country:	Denmark
Application No.	PA 2002 01098
Filing Date:	July 13, 2002

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# Kongeriget Danmark

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Title: Therapeutic TB vaccine.

IPC: A 61 K 39/04; C 07 K 14/35

This is to certify that the attached documents are exact copies of the above mentioned patent application as originally filed.



Patent- og Varemærkestyrelsen  
Økonomi- og Erhvervsministeriet

13 February 2008

Bo Z. Tidemann

13 JULI 2002

Modtaget

## Therapeutic TB vaccine

### Field of invention

The present invention discloses a therapeutic vaccine against latent or active tuberculosis infection caused by the tuberculosis complex microorganisms (*Mycobacterium tuberculosis*., *M.bovis*, *M.africanum*). The invention furthermore discloses a multi-phase vaccine which can be administered either prophylactically or therapeutically as well as a diagnostic reagent for the detection of latent stages of tuberculosis.

### General Background

Human tuberculosis caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) is a severe global health problem, responsible for approx. 3 million deaths annually, according to the WHO. The worldwide incidence of new tuberculosis (TB) cases had been falling during the 1960s and 1970s but during recent decades this trend has markedly changed in part due to the advent of AIDS and the appearance of multidrug resistant strains of *M. tuberculosis*.

Organisms of the tuberculosis complex can cause a variety of diseases, but the commonest route of invasion is by inhalation of bacteria. This initiates an infection in the lung, which can ultimately spread to other parts of the body. Normally, this infection is restricted in growth by the immune system, so that the majority of infected individuals show few signs apart from cough and fever, which eventually abates. Approximately 30% of individuals are unable to contain the infection and they will develop primary disease, which in many cases will eventually prove fatal. However, it is believed that even those individuals who apparently control the infection remain infected, probably for the rest of their life. Certainly, individuals who have been healthy for years or even decades can suddenly develop tuberculosis, which has proven to be caused by the same organism they were infected with many years previously. *M. tuberculosis* and other organisms of the TB complex are unique in that the mycobacteria can evade the immune response and survive for long periods in a refractory non-replicating or slowly-replicating stage. This is referred to as latent TB and is at present a very significant global health problem which is estimated to affect approximately 1/3 of the worlds population (Anon., 2001).

The course of a *M. tuberculosis* infection runs essentially through 3 phases, as illustrated in figure 1. During the acute phase, the bacteria proliferate in the organs, until the immune response increases to the point at which it can control the infection, whereupon the bacterial load peaks and starts declining. After this, a latent phase is established where the bacterial load is kept stable at a low level. In this phase *M. tuberculosis* goes from active multiplication to dormancy, essentially becoming non-replicating and remaining inside the granuloma. In some cases, the infection goes to the reactivation phase, where the dormant bacteria start replicating again. The full nature of the immune response that controls latent infection and the factors that lead to reactivation are largely unknown. However, there is some evidence for a shift in the dominant cell types responsible. While CD4 T cells are essential and sufficient for control of infection during the acute phase, studies suggest that CD8 T cell responses are more important in the latent phase. It is also likely that changes in the antigen-specificity of the response occur, as the bacteria modulates gene expression during its transition from active replication to dormancy.

The only vaccine presently available for clinical use is BCG, a vaccine whose efficacy remains a matter of controversy. Although BCG consistently performs well in animal models of primary infection, it has clearly failed to control the TB epidemic. Consistent with that, BCG vaccination appears to provide protection against pediatric TB (which is due to primary infection), while offering little or no protection against adult disease (which is often reactivation of latent infection acquired in childhood). It has also been shown that vaccination of individuals who are currently sensitized to mycobacteria or latently infected is ineffective. Thus, current vaccination strategies, while effective against primary disease, fail to activate immune responses which efficiently control surviving dormant bacteria.

At this point no vaccine has been developed that confers protection against reactivation whether given as a prophylactic vaccine prior to infection or as a therapeutic vaccine given to already latently infected individuals.

This makes the development of a new and improved vaccine against TB an urgent matter, which has been given a very high priority by the WHO. Many attempts to define protective mycobacterial substances have been made, and different investigators have reported increased resistance after experimental vaccination. However, these efforts have almost exclusively focused on the development of prophylactic vaccines for the prevention of dis-

ease (Doherty, 2002), and such vaccines have not been demonstrated to work if given in a immunotherapeutic fashion (Turner, 2000).

It has been suggested that the transition of *M. tuberculosis* from primary infection to latency is accompanied by changes in gene expression (see, for example, Honer zu Bentrup, 2001, which is incorporated herein by reference). *In vitro* hypoxic culture conditions, which mimic the conditions of low oxygen tension and restricted nutrients found in the granuloma (the location of the latent infection), have been used to analyse changes in gene expression and a number of antigens have been found that are induced or markedly upregulated under these conditions eg. the 16 kDa antigen  $\alpha$ -crystallin (Boon, 2001, Monahan, 2001, Florczyk 2001, Sherman 2001, Manganeli, 2001, all of which are incorporated herein by reference).

In this invention we show that these molecules can be used as the basis for therapeutic vaccines, whereas vaccines which are designed to elicit protective immune responses prior to infection (prophylactic vaccination) are only effective against primary infection. The immune responses elicited are powerless against the latent stage of the disease, because the bacteria have changed the antigens that they produce so that in essence they have altered their appearance and the immune system can no longer recognize them. However, latency is a dynamic process, maintained by the immune response, as indicated by the dramatic increase in the risk of reactivation of TB after HIV infection or other events that compromise immunity. Therefore, an effective vaccination strategy to protect infected individuals (therapeutic vaccination) is possible, but only if it is directed against those antigens expressed in the latent stage.

Further, since TB vaccines do not result in sterilizing immunity but rather control the infection at a subclinical level (thereby resulting in the subsequent establishment of latent infection), a multiphase vaccine which combines components with prophylactic and therapeutic activity is described in this invention. After conventional prophylactic vaccination, the evasion of the primary immune response and the subsequent development of latent disease is probably at least in part due to the change in the antigenic profile of the invading bacteria. Thus, vaccinating with antigens associated with latent TB should prevent or reduce the establishment of latent infection and therefore, a vaccine incorporating antigens expressed by the bacteria both in the first logarithmic growth phase and during latent disease should improve long-term immunity when used as a prophylactic vaccine. Such a multiphase vaccine will obviously also be efficient as a therapeutic vaccine thereby addressing the problem that the majority of

the population in the third world who would receive a future TB vaccine would be already latently infected.

For a number of years, a major effort has been put into the identification of protective antigens for the development of novel prophylactic vaccines against TB and today a few antigens with demonstrated protective activity in prophylactic vaccines have been identified (eg. ESAT-6, the 38 kDa antigen, Ag85A and Ag85B). Such molecules would be components, which in combination with latency associated antigens such as  $\alpha$ -crystallin, could form a multiphase vaccine.

As noted in the references cited above, it is already known that some genes are upregulated under conditions that mimic latency. However, these are a limited subset of the total gene expression during latent infection. Moreover, as one skilled in the art will readily appreciate, expression of a gene is not sufficient to make it a good vaccine candidate. The only way to determine if a protein is recognized by the immune system during latent infection with *M. tuberculosis* is to produce the given protein and test it in an appropriate assay as described herein. Of the more than 200 hundred antigens known to be expressed during primary infection, and tested as vaccines, less than a half dozen have demonstrated significant potential. So far only one antigen has been shown to have any potential as a therapeutic vaccine (Lowrie, 1999). However this vaccine only worked if given as a DNA vaccine, an experimental technique so far not approved for use in humans. Moreover, the technique has proved controversial, with other groups claiming that vaccination using this protocol induces either non-specific protection or even worsens disease (Turner, 2000). In contrast, the antigens described in the invention may be incorporated in vaccines that use well-recognized vaccination technology, as demonstrated in provided examples.

Finally, the immunodominant antigens identified in this invention may be used as diagnostic reagents. Our group has abundantly demonstrated that antigens expressed by mycobacteria during the early stages of the infection, such as ESAT-6 (Early Secretory Antigen Target-6) are recognized in individuals who are in the process of developing primary TB, even though they are healthy at the time of diagnosis (Doherty 2002). However, the large number of contacts who are exposed, and almost certainly infected, remain negative to this antigen (Doherty 2002). Since those individuals latently infected remain healthy by making an immune response against the latent bacteria, they must be making an immune response to those antigens expressed by the latent bacteria. Thus, the antigens of the

invention may also be used to diagnose latent infection and differentiate it from primary acute TB.

## Summary of the invention

The invention is related to preventing, treating and detecting infections caused by species of the tuberculosis complex (*Mycobacterium tuberculosis*, *M. bovis*, *M. africanum*) by the use of a polypeptide comprising a *M. tuberculosis* antigen or an immunogenic portion or other variant thereof, or by the use of a DNA sequence encoding a *M. tuberculosis* antigen or an immunogenic portion or other variant thereof. The invention discloses a new therapeutic vaccine against tuberculosis comprising antigens induced during the latent stage of TB-infection. It also discloses a multiphase vaccine incorporating a combination of prophylactic and therapeutic antigens as well as diagnostic reagents for the detection of the latent stage of *M. tuberculosis* infection.

## Detailed disclosure of the invention

The present invention discloses the use of one or more polypeptides, nucleic acids encoding these polypeptides or fragments hereof, which polypeptides are expressed during the latent stage of the mycobacterial infection, which stage is characterized by low-oxygen tension in the microenvironment of the mycobacteria, for a therapeutic vaccine against tuberculosis.

The polypeptides comprises one or more amino acid sequences selected from

- (a) The sequences presented in Table 1.
- (b) an immunogenic portion, e.g. a T-cell epitope, of any one of the sequences in (a);  
and /or
- (c) an amino acid sequence analogue having at least 70% sequence identity to any one of the sequences in (a) or (b) and at the same time being immunogenic.



Table 1. Amino acid sequences of selected low oxygen induced antigens		
Rv no.	Seq ID no.	Sequence
Rv0079	1	VEPKRSRLVVCAPEP SHAREFPD VAVFSGGRANASQAERLARAVGRVLADRGVTGG ARVRLTMANCADGPTLVQINLQVGDTP LRAQAATAGIDDLR PALIRLDRQIVRAS A QWCPRPWPDRPRRRLTPAEALVTRRKPVVLRRA TPLOAIAAMDAMDYDVHLFTDA ETGEDAVVYRAGPSGLRLARQHVFPPGWSRCRAPAGPPVPLIVNSRPTPVLTEAA AVDRAREHGLPFLFFTDQATGRGQLLYSRYDGNLGLITPTGDGVADGLA
Rv0080	2	MSPGSRRAS PQSAREVV ELDRDEAMRL LASVDHGRVVFTRAALPAIRPVNHLVVDG RVIGRTRLTAKVSVAVRSSADAGVVVAYEADDLP RRRTGWSVVVTGLATEVSDPE QVARYQRL LHPVWNMAMDTVVAIEPEIVTGIRIVADS RTP
Rv0081	3	VESEPLYKLKAEFFKTLAHPARIRILELLIVERDRSVGELLSSDVGLESSNLSQQLG VLRRAGVVAARRDGNAMIYSIAAPDIAELLAVARKVLARVLSDRVAVLEDLRAGG- SAT
Rv0363c	4	MPIATPEVYAEMLGQAKQNSYAFPAINCTSSETVNAAIKGFADAGSDGIIQFSTGG AEFGSGLGVKDMVTGAVALAEFTHVIAAKYPVNVALHTDHCPKDKLDSYVRPLLAI SAQRVSKGGNPLFQSHMWDGSAVPIDENLAI AQELLKAAAAAKIILEIEIGVVGGE EDGVANEINEKLYTSPEDFEKTIEALGAGEHGKYL LAATFGNVHGVYKPGNVKL RP DILAQQQQVAAAKLGLPADAKPFD FVFHGGSGSLKSEIEEALRYGVVKMNVDTDTQ YAFTRPIAGHMFNTYDGV LKVDGEVGVKKVYDPRS YLKKA EASMSQRVVQACNDLH CAGKSLTH
Rv0569	5	MKAKVGDWLVIKGATIDQPDHRLG LIEVRSSDGSPPYVVRWLET DHVATVIPGPDA VVVTAE EQNAADERAQH RFGAVQSAILHARGT
Rv0572c	6	MGEHAIKRHRMRQRKPTKHPLAQKRGARILVFTDDPRRSVLIVPGCHLDSMRREKNA YYFQDGNALVGMVVS GGTVEYDADDRTYV VQLTDGRHTTESSFEHSSPSRSPQS- DDL
Rv0574c	7	VAGNPDVVTVLLGGDVMLGRGVDQILPHPGKPQLRERYMRDATGYVRLAERVNGRI PLPVDWRWPWGEALAVLENTATDVCLINLETTITADGEFADRKPVCYRMHPDNVPA LTALRPHVCALANNHILDFGYQGLTDTVAALAGAGIQSVGAGADLLAARRSALVTV GHERRVIVGSVAAESSGVPE SWAARRDRPGVWLIRDPAQRDVADDVAAQVLADKRP GDIAIVSMHWGSNWGYATAPGDVAFHRLIDAGIDMVHGHSSHHPRIE IYRGKPI LYGCGDVDDYEGIGGHESFRSELRLLYLTVD PASGNLISLQMLPLRVSRMRLQR ASQTDTEWLRNTIERISRRFGIRVVTRPDNLLEVVPANLTSKE
Rv1264	8	VTDHVREADDANIDDLGLDGGTARAERAKLVEWLLEQGITPDEIRATNPPLLLAT RHLVGDDGTYSAREISENYGV DLELLQRVQRAVGLARVDDPDVAVHMRADGEAAA RAQRFVELGLNPDQVVLVVRVLA EGLSHAAEAMRYTALEAIMRPGATELDIAKGSQ ALVSQIVPLLGPMIQDMLFMQLRHMMETEAVNAGERAAGKPLPGARQVTVAFADLV GFTQLGEVVS AEELGHLAGRLAGLARDLTAPPVWF IKTIGDAMVLC PDPAPLLDT VLKLVEVVDTDNNFPRLRAGVASGMAVSRAGDWF GSPVNVASRVTVARPGAVLVA DSVREALGDAPEADGFQWSFAGPRLRGIRGDVRLFRVRRGATRTGSGGAAQDDD- LAGSSP
Rv1592c	9	MVEPGNLAGATGAEWIGRPPHEELQRKVRPLLPSDDPFYFPAGYQHAVPGTVLRS RDVELAFMGLIPQPV TATQLLYRTTNMYGNPEATVTTVIVPAELAPGQTCPLLSYQ CAIDAMSSRCFPSYALRRRAKALGSLTQMELLMISAALAEGWAVSPDHEGPKGLW GSPYEPGYRVL DGIRAA LNSERVGLSPATPIGLWGYSGGGLASAWAAEACGEYAPD LDIVGAVLGSPVGD LGHTFRRLNGTLLAGLPALVVAALQHSYPGLARV I KEHANDE GRQLEQLTEMTTVDAVIRMAGRDMGDFLDEPLEDILSTPEISHVFGDTKLGSAPV TPPVLIQAVHDY LIDVSDIDALSYTAGGANV TYHRDLFSEHVS LHPLSAPMTL RWLTD RFAAGKPLTDHRVRTTWTPTIFNPMTYAGMARLAVIAAKVITGRKLSRRPL
Rv1733c	10	MIATTRDREGATMITFRLRLPCRTILRVFSRNPLVRGTDRLEAVVMLLAVTVSLT IPFAAAAGTAVQDSRSHVYAHQAQTRHPATATVIDHEGVIDSNTTATSAPPRTKIT VPARWVVNGIERSGEVNAKPGTKSGDRVGIWVDSAGQLVDEPAPPARAIADAALAA LGLWLSVA AVAGALLALTRAILIRVRNASWQH DIDSLEFCTQR

Table 1. (continued) Amino acid sequences of selected low oxygen induced antigens		
Rv no.	Seq ID no.	Sequence
Rv1734c	11	MTNVGDQGVDAVFGVIYPPQVALVSFGKPAQRVCAVDGAIHVMTTVLATLPADH GCSDDHRGALFFLSINELTRCAAVTG
Rv1736c	12	VTVTPRTGSRIEELLARSGRFFIPGEISADLRTVTRRGGRDGDVFYRDRWSDHK VVRSTHGVNCTGSCSWKIYVKDDIITWETQETDYPVGPDRPEYEPGCGPRGAA FSWYTYSPTRVRHPYARGVLVEMYREAKARLGDPVAAWADIQADPRRRRRYQRA RGKGGGLVRVSWAEATEMIAAAHVHTISTYGPDRVAGFSPIPAMSMVSHAAGSRF VELIGGVMTSFYDWYADLPVASPQVFGDQTDVPESGDWWDVVWQCASVLLTYPN SRQLGTAEELLAHIDGPAADLLGRTVSELRRADPLTAATRYVDTFDLRGRATLY LTYWTAGDTRNRGREMLAFAQTYRSTDVAPPRGETPDFLPVVLEFAATVDPEAG RRLLSGYRVPIAALCNALTEALPYAHTVAAVCRTGDMMGELFWTVVVPYVTMTI VAVGSWWRYRYDKFGWTRSSQLYESRLLRIASPMFHFGLVVIIVGHGIGLVIP QSWTQAAGLSEGAYHVQAVVLGSIAGITTLAGVTLLIYRRRTRGPFVFMATTVND KVMYLVLVAAIVAGLGATALGSGVGEAYNYRETVSVWFRSVWVLQPRGDLMAE APLYYQIHVLIGLALFALWPFTRLVHAFSAPIGYLFRPYIIYRSREELVLTR- PRRRGW
Rv1737c	13	MRGQAANLVLATWISVVNFWAWNLIPLSTSYARDMSLSSAEASLLVATPIILVG ALGRIVTGPLTDRFGGRAMLIAVTLASILPVLAVGVAATMGSYALLVFFGLFLG VAGTIFAVGIPFANNWYQPARRGFSTGVFGMGVGTALSFFTPTFRVWFGLFT THAIVAAALASTAVVAMVLRDAPYFRPNADPVLPRLKAAARLPVTWEMSFLYA IVFGGFVAFSNYLPTYITTIYGFSTVDAGARTAGFALAAVLARPVGGWLSDRIA PRHVVLASLAGTALLAFAAALQPPPEVWSAATFITLAVCLGVGTGGVFVAWVARR APAASVGSVTGIVAAAGGLGGYFPPLVMGATYDPVDNDYTVGLLLLVALATLVAC TYTALHAREPVSEEASR
Rv1738c	14	MCGDQSDHVLQHWTVDISIDEHEGLTRAKARLRWREKELVGVGLARLNPADRN PEIGDELSVARALSDLGKRLKVSTHDI EAVTHQPARLLY
Rv1739c	15	MIPTMTSAGWAPGVVQFREYQRRWLRGDVLAGLTVAAYLIPQAMAYATVAGLPP AAGLWASIAPLAIYALLGSSRQLSIGPESATALMTAAVLAPMAAGDLRRYAVLA ATLGLLVGLICLLAGTARLGLASLRSPVLVGYMAGIALVMISSQLGTITGTS VEGNEFFSEVHSFATSVTRVHWPTFVLAMSVLALLTMLTRWAPRAPGPPIIAVLA ATMLVAVMSLDAKGIAIVGRIPSGLPTPGVPPVSVEDLRALIIPAAGIAIVTFT DGVLTARAFARRGQEVNANAELRAVGACNIAAGLTHGFPVSSSSSRTALADV GGRTQLYSLIALGLVIVMVFAAGLLAMFPIAALGALVVYAALRLIDLSEFRRL ARFRRSELMLALATTAAVLGLGVFYGVLAVALSILELLRRVAHPHDSVLGFVP GIAGMHIDIDDYPOAKRVPGLVVYRYDAPLCFANAEDFRRRALTVDQDPGQVEW FVLNAESNVEVDLTALDALDQLRTELLRRGIVFAMARVKQDLRESLRAASLLDK IGEDHIFMTLPTAVQAFRRR
Rv1813c	16	MITNLRRTAMAAAGLGAALGLGILLVPTVDAHLANGSMSEVMMSEIAG LPIPPIIHYGAIAYAPSGASGKAWHQRTPARAEQVALEKCGDKTCKVVS RFTRCGAVAYNGSKYQGGTGLTRRAAEDDAVNRLEGGRIVNWACN

Table 1. (continued) Amino acid sequences of selected low oxygen induced antigens

Rv no.	Seq ID no.	Sequence
Rv1997c	17	LSASVSATTAHHGLPAHEVVLLLESDFYHGLSDGEAAQRLERFGPNTLAVVTRASL LARILRQFHHPLIYVLLVAGTITAGLKEFVDAAVIFGVVVINAIVGFIQESKAEAA LQGLRSMVHTHAKVVREGHEHTMPSEELVPGDLVLLAAGDKVPADLRLVRQTGLSV NESALTGESTPVHKDEVALPEGTPVADRRNIAYSGLTVTAGHGAGIVVATGAETEL GEIHRLVGAAEVVATPLTAKLAWFSKFLTIAILGLAALTFGVGLLRRQDAVETFTA AIALAVGAIPEGLPTAVTITLAIGMARMAKRRRAVIRRLPAVETLGSTTVICADKTG TLTENQMTVQSIWTPHGEIRATGTGYAPDVLLCDTDDAPVPVNNANAALRWSLLAGA CSNDAALVRDGTWRQIVGDPTEGAMLVVAAGAGFNPERLATTLPOVAAIPFSSERO YMATLHRDGTDHVVLAKGAVERMLDLCGTEMGADGALRPLDRATVLRATEMLTSRG LRVLATGMGAGAGTPDDFDENVIPGSLALTGLQAMSDPPRAAASAVAACHSAGIA VKMITGDHAGTATAIATEVGLLDNTEPAAGSVLTGAELAALSADQYPEAVDTASVF ARVSPEQKLRLVQALQARGHVAMTGDGVNDAPALRQANIGVAMGRGGTEVAKDAA DMVLTDDDFATIEAAVEEGRGVFDNLTKFITWTLPNTLGEGLVILAAIAVGVALPI LPTQILWINMTTALGLMLAFEPKEAGIMTRPPRPDPQPLLTGWLVRRTLLVSTL LVASAWWLFWEWDNGAGLHEARTAAALNLFVVVEAFYLFSCRSLTRSAWRLGMFAN RWIILGVSAQAIQAFAITYLPAMNMVFDTPIDIGVWVRIFAVATAITIVVATDTL LPRIRAQPP
Rv1998c	18	MSFHDLLHHQGVPPFVLNPAWDVPSALAYLAEGFTAIGTTSFGVSSSGHPDGHRATR GANIALAAALAPLQCYVSDIEDGYSDEPDIAIDYVAQLSTAGINIEDSSAEKLID PALAAAKIVAIAKQRNPEVFNARVDTYWLRQHADTTSTIQALRYVDAGADGVFVP LANDPDELAELTRNIPCVNTLPVPGTLTIADLGELGVARVSTGSPVYSAGLYAAAH AARAVSDGEQLPRSPVYAEQLQARLVLDYENRTSTT
Rv2003c	19	VVKRSRATRLSPSIWSGWESPQCRSIRARLLLLPRGRSRPPNADCCWNQLAVTPDTR MPASSAAGRDAAYDAWYDSTPTGRPIILATEVAALRPLIEVFAQPRLEIGVGTGRFA DLLGVRFGLDPSRDALMFARRRGVLVANAVGEAVPFVSRHFGAVLMAFTLCFVTD AAIFRETRRLADGGGLVIGFLPRGTPWADLYALRAARGQPGYRDARFYTAEELEQ LLADSGFRVIARRCTLHQPPGLARYDIEAAHDGIQAGAGFVAISAVDQAHEPKD- DHPLESE
Rv2005c	20	MSKPRKQHGTVVGVGDSLESDAACWGATDAAMRNIPLTVVHVVNADVATWPPMPY PETWGVWQEDEGRQIVANAVKLAKAEVAGDRKLSVKSELVSTPVPMTVEISNEAE MVVLGSSSGRGALARGLLGSVSSSLVRRACCPVAVIHSDDAVIPPQHPVLVIGIDG SPVSELATAVAFDEASRRGVELIAVHAWSDVEVVELPGLDFSAVQQEAELSLAERL AGWQERYPDVPVSRVVCDRPAKRLVQKSASASQLVVVGSHGRGGLTGMLLGSVSNA VLHAARVPVIVARQS
Rv2007c	21	VTYVIGSECVDVMDKSCVQECVPDCIYEGARMLYINPDECVDGACKPACRVEAIY WEGDLPDDQHQLGDNAFFHQVLPGRVAPLGSPPGAAAVGPVIGVDTPLVAAIP- VECP
Rv2028c	22	MNQSHKPPSIVVGIDGSKPAVQAALWAVDEAASRDIPRLRLLYAIEPDDPGYAAHGA AARKLAAAENAVRYAFTAVEAADRPVKVEVEITQERPVTSLIRASAAAALVCVGAI GVHHFRPERVGSTAAALALSAQCPVAIVRPHRVPIGRDAAWIVVEADGSSDIGVLL GAVMAEARLRDSPVRVVTCTQSGVGDGTGDDVRASLDRLARWQPRYPDVRVQSAV HGELLDYLAGLGRSVHVMVLSASDQEHVEQLVGAPGNAVLEAGCTLLVVGQQYL
Rv2029c	23	MTEPAWDEGKPRIITLTMPALDITTSVDVVRPTEKMRGAPRYDPGGGGINVAR IVHVLGGCSTALFPAGGSTGSLMALLGDAGVPFRVPIAASTRESFTVNESRTAK QYRFVLPGPSLTVAEQEQCLDELRGAAASAAFFVASGSLPPGVAADYYQRVADICR RSSTPLILDTSGGGLQHISSGVFLKASVRELRECVGSELLTEPEQLAAAHELIDR GRAEVVVVSLGSQGALLATRHASHRFSSIPMTAVSGVGAGDAMVAAITVGLSRGWS LIKSVRLGNAAGAAMLLTPGTAAACNRDDVERFFELAAEPTEVGQDQYVWHPIVN- PEASP

Table 1. (continued) Amino acid sequences of selected low oxygen induced antigens

Rv no.	Seq ID no.	Sequence
<b>Rv2030c</b>	<b>24</b>	VLMTAAADVTRRSPPRVFRDRREAGRVLAELLAAYRDQPDVIVLGLARGGLPVAVE VAAALHAPLDAFVVRKLGAPGHDEFVAVGALASGGRVVVNDVVRGLRITPQQLRDI AEREGRELLRRRESAYRGERPPTDITGKTVIVVDDGLATGASMFAAVQALRDAQPAQ IVIAVPAPESTCREFAGLVDDVVCATMPTPFLAVGESFWDFRQVTDEEVRRLLAT PTAGPSLRRPAASTAADVLRRAIDAPGGVPTHEVLAELVGDARIVLIGESSHGTH EFYQARAAMTQWLIEEKGFAGAVAAEADWPDAYRVNRYVRGLGEDTNADEALSGFER FPAMWVRNTVVRDFVEWLRTNRQRYESGALRQAGFYGLDLYSLHRSIQEVISYLDK VDPRAAARARARYACFDHACADDGQAYGFAAAGAGPSCEREAVEQLVDVQRNALA YARQDGLLADELFFAQQNAQTVRDAEVYVRAMFSGRVTSWNLRDQHMAQTLGSL THLDRHLDAPPARIVVWAHNSHVGDARATEVWADGQTLGQIVRERYGDESRSIGF STYTGTVTAASEWGGIAQRKAVRPALHGSVEELFHQTADSFLVSARLSRDAEAPLD VVRLGRAIGVVYLPATERQSHYLHVRPADQFDAMIHIDQTRALEPLEVTSRWIAGE NPETYPTGL
<b>Rv2031c</b>	<b>25</b>	MATTLPVQRHPRSLFPEFSELFAPPSFAGLRPTFDTRLMRLEDEMKEGRYEV- RAELPGVDPDKDVIDMVRDGLTIKAERTEQKDFDGRSEFAYGSFVRTVSLPVGA- DEDDIKATYDKGILTVSVAVSEGKPTKHIQIRSTN
<b>Rv2032</b>	<b>26</b>	MPDTMVTDDVIKSAVQLACRAPSLHNSQPWRWIAEDHTVALFLDKDRVLYATDHSG REALLGCGAVLDHFRVAMAAAGTTANVERFPNPNDFLHLASIDFSPADFVTEGHR RADAILLRRTDRLPFAEPDWDLVESQLRTTVTADTVRIDVIADMRPELAAASKL TESLRLYDSSYHAELFWWTGAFETSEGIPHSSLVSAAESDRVTFGRDFPVVANTDR RPEFGHDSKVLVLSTYDNERASLLRCGEMLSAVLLDATMAGLATCTLTHTITELHA SRDLVAALIGQPATPQALVRVGLAPEMEEPPPATPRRPIDEVFHVRADHR
<b>Rv2428</b>	<b>27</b>	MPLLTIGDQFPAYQLTALIGGDLKVDKQPGDYFTTITSDEHPGKWRVVFVWPKD FTFVCPTEIAAFSKLNDEFEDRDAQILGVSIDSEFAHFQWRAQHNDLKTLPFPMLS DIKRELSQAAGVLNADGVADRVTFIVDPNNEIQFVSATAGSVGRNVDEVLRVLDAL QSDELCACNWRKGDPTLDAGELLKASA
<b>Rv2623</b>	<b>28</b>	MSSGNSSLGIIVGIDDSAPAAQVAVRWAAARDAELRKIPLTLVHAVSPEVATWLEVP PPGVLRWQQDHGRHLIDDALKVVEQASLRAGPPTVHSEIVPAAAVPTLVDMKDAV LMVVGCLGSGRWPGRLLGSVSSGLLRHAHCPVVIHDEDSVMPHPQAPVLVGVGD SSASELATAIAFDEASRRNVDLVALHAWSDVDVSEWPGIDWPATQSMAEQVLAERL AGWQERYPNVAITRVVVRDQPARQLVQRSEEAQLVVVSGRGRGGYAGMLVGSVGET VAQLARTPVIVARESIT
<b>Rv2624c</b>	<b>29</b>	MSGRGEPTMKTIIVGIDGSHAAITAALWGVDEAISRAVPLRLVSVIKPHTHPSDDY DRDLAHAERSLREAQSAVEAAGKLVKIETDIPRGPAGPVLVEASRDAEMICVGSVG IGRYASSILGSTATELAEKACHPCVAVMRSKVDQPASDINWIVVRMTDAPDNEAVLE YAAREAKLRQAPILALGGRPEELREIPDGEFERRVQDWHHRHPDVRVYPITHTGT ARFLADHDERVQLAVIGGGEAGQLARLVGPSGHPVFRHAECVSLVVR
<b>Rv2625c</b>	<b>30</b>	MRDAIPLGRIAGFVVNVHWSVLVILWLFVWLSLATMLPGTVGGYPVVVYWLLGAGGA VMLLASLLAHELAAHVARRAGVSVESVTLWLFGGVTALGGEAKTPKAAFRIFAAG PATSLALSATFGALAITLAGVRTPAIVISVAWWLATVNLLGLFNLLPGAPLDGGR LVRAYLWRRHGDVVRAGIGAARAGRVVALVLIAGLAEFVAGGLVGGVWLAFIGWF IFAAAREEETRISTQQLFAGVRVADAMTAQPHATPGWINVEDFIQRYVLGERHSAY PVADRDGSITGLVALRQLRDVAPSRRTTSVGDIALPLHSVPTARPQEPLTALLER MAPLGPRSRALVTEGSAAVVGIVTPSDVARLIDVYRLAQPEPTFTTSPQADRFSDAG
<b>Rv2626c</b>	<b>31</b>	MTTARDIMNAGVTCVGEHETLTAAQYMRHHDIGALPICGDDRLHGMILTDRDIVI KGLAAGLDPNATAGELARDSIYYVDANASIQEMLNVMEHQVRRVPVISEHRLVG IVTEADIARHLPEHAIVQFVKAICSPMALAS

Table 1. (continued) Amino acid sequences of selected low oxygen induced antigens		
Rv no.	Seq ID no	Sequence
Rv2727c	32	MASSASDGTHERSAFRLSPVLSGAMGPFMHTGLYVAQSWRDYLGQQPDKLPPIARP TIALAAQAFRDEIVLLGLKARRPVSNHRVFERISQEVAAGLEFYGNRRWLEKPSGF FAQPPPLTEVAVRKVKDRRRSFYRIFDSDGFTPHPGEPGSQRWLSYTANNREYALL LRHPEPRPWLVCVHGTEMGRAPLDLAVFRAWKLHDELGLNIVMPVLPMHGPRGQGL PKGAVFPGEDVLDDVHGTAQAVWDIRLLSWIRSQEEESLIGLNGLSLGGYIASLV ASLEEGLACAILGVPVADLIELLGRHCGLRHKDPRRHVTVMKAEPIGRMISPLSLTP LVPMPGRFIYAGIADRLVHPREQVTRLWEHWGKPEIVWYPGGHTGFFQSRPVRRFV QAALQSGLLDAPRTQDRSA
Rv2628	33	MSTQRPRHSGIRAVGPIYAWAGRCGRIGRWGVHQEAMMNLAIWHPRKVQSATIIYQVT DRSHDGR TARVPGEITSTVSGWLSELGTQSPLADELARAVRIGDWPAAYAIGEHL SVEIAVAV
Rv2629	34	MRSERLRWLVAEEGPFASVYFDDSDHTLDAVERREATWRDVRKHLESRDQKQELID SLEEAVRDSRPAVGQRGRALITGEQVLVNEHLIGPPPATVIRLSDYPIYVPLIDL EMRRPTYVFAAVDHTGADVLYQGATISSTKIDGVGYPVHKPVTAGWNGYGFQHT TEEAIRMNCRADVADHLTRLVDAADPEVVFVSGEVRSRTDLLSTLPQRVAVRVSQHL AGPRKSALDEEIIWDLTSAEFTRRRYAEITNVAQQFEAEIGRGSGLAAQGLAEVCA ALRDGDVDTLIVGELGEATVVTGKARTTVARDADMLSELGEPVDRVARADEALPFA AIAVGAALVRDDNRIAPLDGVDGALLRYAATNRLGSHRS
Rv2630	35	MLHRDDHINPPRPRGLDVPCARLRATNPLRALARCVQAGKPGTSSGHRVPHATDL RIEAWAPTRDGCIRQAVLGTVESFLDLESAAVHTRLRLTADRDDLLVAVLEEVI YLLDTVGETPVDLRLRDVDGGVDVTFATTDASTLVQGVAVPKAVSLNELRFSQGR HGWRCVATLDV
Rv2659c	36	VTQTGKRQRRKFGRIRQFNSGRWQASYTGPDGRVYIAPKTFNAKIDAEAWLTDRRR EIDRQLWSPASGQEDRPGAPFGEYAEGLKQKQGIKDRTRAHYRKLNDNHILATFAD TDLRDITPAAVRRWYATTAVGTPTMRAHSYSLLRIMQ TALADDLIDSNPCRISGA STARRVHKIRPATLDELETITKAMPDPYQAFVLMAAWLMRYGELTELRRKDIDLH GEVARVRRRAVVRVGEFGKVTTPKSDAGVRDISIPPHLIPAIEDHLHKHVNPGRESL LFPSVNDPNRHLAPSALYRMFYKARKAAGRPDRLRVHDLRHSAGVLAASTGATLAEL MQRLGHSTAGAALRYQHAAGKRDREIAALLSKLAENQEM
Rv2780	37	MRVGIPETETKNEFRVAITPAGVAELTRRGHEVLIQAGAGEGSAITDADFKAAGAQ LVGTADQVWADADLLLKVKEPIAAEYGRRLRHGQILFTFLHLAASRACTDALLDSGT TSIAYETVQTADGALPLLAPMSEVAGRLAAQVGAYHLMRTQGGRGVLMGGVPGVEP ADVVVIGAGTAGYNAARIANGMGATVTVLDINIDKLRLDAEFCEGRIHTRYSSAYE LEGAVKRADLVIGAVLVPGAKAPKLVNSSLVAHMKPGAVLVDIAIDQGGCFEGSRP TTYDHPTFAVHDTLFCYVANMPASVPKTSYALTNTATMPYVLELADHGWRACRSN PALAKGLSTHEGALLSERVATDLGVFPFTEPASVLA
Rv3126c	38	MVIRFDQIGSLVLSMKSLASLSFQRCLENSSLVAALDRDLDAAVDELSALSFDALT TPERDRARRDRDHPWSRSRSQLSPRMAHGAVHQCQWPKAVWAVIDNP
Rv3127	39	VLKNAVLLACRAPSVHNSQPWRWVAESGSEHTTVHLFVNRHRTVPATDHSGRQAI I SCGAVLDHLRIAMTAAHWQANITRFPQPNQPDQLATVEFSPIDHVTAGQRNRAQAI LQRRTDRLPFDSMPYWHLFEPALRDAVDKDVAMLDVVSDDQRTLRVVASQLSEVLR RDDPYHAELEWWTSPFVLAHGVPDPTLASDAERLRVDLGRDFPVRSYQNRRAELA DDRSKVLVLSTPSDTRADALRCGEVLSTILLECTMAGMATCTLTHLIESSDSRDIV RGLTRQGEPPQALIRVGIAPPLAAMPAPTTPRRPLDSVLQIRQTPEKGRNASDRNAR ETGWFSP
Rv3128c	40	VVSASGGQCGKYLAASMLQLDGLERHGVLEFGRDRYGPEVREELLAMSAASIDRY LKTAKAKDQISGVSTTKPSPLLRNSIKVRRAGDEVEAEPGFFEGDTHVACGPTLKG EFAHTLNLTDVHIGWVTRTVRNARHILAGLKASVTEIPHGITGLDFDNGTVFL NKPVISWAGDNGI - YFTRFRPYKKNH*ATIESKNNHLVRKYAFYYRYDTAEERAVLNRMWKLVDNRLNYL TPTIKPIGYASSADGRRRLYDAPQTPDRPLAARVLSAAQQADLITYRDSLNPQ IGRKIADLQNRLLILAKEKTEQLYLANIPTALPDIHKGILIKAG
Rv3129	41	VVQGRTVLFRTAEGAKLFSVAKCAVAFEADDHNVAEGWSVIVKVRAQVLTDDAGV REAERAQLLPWTATLKRHCVRVIPWEITGRHFRFGPEPDRSQTFACEASSHNQR

Table 1. (continued) Amino acid sequences of selected low oxygen induced antigens		
Rv no.	Seq ID no.	Sequence
Rv3130c	42	MNHLTTLDAGFLKAEDVDHRVSLAIGALAVIEGPAPDQEAFLSSLAQRLRPCTRFG QRLRLRPFDLGAPKWDDPDFDLGRHVWRIALPRPGNEDQLFELIADLMARRLDRG RPLWEVWVIEGLADSKWAILTKLHHCMAADGIAATHLLAGLSDESMDSFASNIHTT MQSQSASVRRGGFRVNPSEALTASTAVMAGIVRAAKGASEIAAGVLSPAASSLNGP ISDLRRYSAAKVPLADVEQVCRKFDVTINDVALAAITESYRNVLIQRGERPRFDSL RTLVPVSTRSNSALSKTNDNRVSLMLPNLPVDQENPLQRLRIVHSRLTRAKAGGQRO FGNTLMAIANRLPFPMATAVAVGLLMRLPQRGVVTATNVPGRPRPLQIMGRRVLDL YPVSPIAMQLRTSVAMLSYADDLYFGILADYDVVADAGQLARGIEDAVARLVAISK RRKVTRRRGALS LVV
Rv3131	43	MNTHFPDAETVRTVLTAVRAPSIHNTQPWRWRVCPTSLELFSRPDMQLRSTDPDG RELILSCGVALHHCVALASLGWQAKVNRFPDPKDRCHLATIGVQPLVPDQADVAL AAAI PRRTDRRAYSCWPVPGDIALMAARAARGVMLRQVSALDRMKAI VAQAVL DHVTDEEYLRELTIIWSGRYGSVAGV PARNEPPSDPSAPIPGRLFAGPGLSQPSDVL PADDGAAILALGTETDDRLARLRAGEAASIVLLTATAMGLACCPITEPLEIAKTRD AVRAEVFGAGGYPQMLLRVGWAPINADPLPPTPRRELSQVVEWPEELLRQRC
Rv3132	44	MTTGGLVDENDGAAMRPLRHTLSQLRLHELLVEVQDRVEQIVEGRDRLDGLVEAML VVTAGLDLEATLRAIVHSATSLVDARYGAMEVHQRHVRVLFVYEGIDEETVRRIG HLPKGLGVIGLLIEDPKPLRLDDVSAHPASIGFPYPYHPPMRTFLGVPVRVRDESFG TLYLTDKTINGQPFSDDEVLVQALAAAAGIAVANARLYQQAKARQSWIEATRDIA ELLSGTEPATVFRVLAEEALKLTAADAALVAVPVDEDMPAADV GELLVIETVGS AV ASIVGRTIPVAGAVLREVFVNGIPRRVDRVDLEGLDELADAGPALLPLRARGTVA GVVVVLSQGGPGAFTDEQLEMAAFADQAALAWQLATSQRMRRELDVLTDRDRIAR DLHDHVIQRLFAIGLALQGAVPHERNPEVQQRSLSDVVDLQDVIQEI RTTIYDLHG ASQGITRLRQRIDAAVAQFADSGLRTSVQFVGPLSVVDSALADQAEAVVREAVSNA VRHAKASTLTVRVKVDDDLCEIVTDNGRGLPDEFTGSGLTNLQRRAEQAGGEFTLA SVPGASGTVLRWSAPLSQ
Rv3133c	45	VVKVFLVDDHEVVRRLVLDLLGADPELDVVGEGSVAEAMARVPAARPDVAVLDVR LPDGNIGIELCRDLLSRMPDLRLCLILTSYTSDEAMLDAILAGASGYVVKDIKGMELA RAVKDVGAGRSLLDNRAAAALMAKLRGAEEKQDPLSGLTDQERTLLGLLSEGLTNK QIADRMFLAEKTVKNYSRLLAKLGMERRTQAAVFATELKRSPPGDGP
Rv3134c	46	MSDPRPARAVVVGIDGSRAATHAALWAVDEAVNRDIPRLVYVIDPSQLSAAGEGG GQSAARAALHDASRKVEATGQPVKIE TEVL CGRPLTKLMQESRSAAMLCVGSVGLD HVRGRRGSVAATLAGSALCPVAVIHPSPAEPATTSQVS AVVAEVDNGVVL RHAFEE ARLRGVPLRAVAVHAAETPDDEVEQGSRLAHVHLSRRLAHWTRLYPEVRVDRAIAGG SACRHLAANAKPGQLFVADSHSAHEL CGAYQPGCAVLTVRSANL
Rv3841	47	MTEYEGPKTKFHALMQEQIHNEFTAAQQYVAIAVYFDS EDPQLAKHFYSQAVEER NHAMMLVQHLLDRDLRVEIPGVDTVRNQFDRPREALALALDQERTVTDQVGRLTAV ARDEGDFLGEQFMQWFLQE QIEEVALMATLVRVADRAGANLFELENFVAREVDVAP AASGAPHAAGGRL
Rv3842c	48	MTWADEVLAGHPFVVAHRGASAAPEHTLAAYDLALKEGADGVECDVRLTRDGH VCVHRRRLDRTSTGAGLVSTMTLAQLRELEYGAWHDSWRPDGSHGDTSLTLTLDAL VSLVLDWHRPVKIFVETKHPVRYGSLVENKLLALLHRFGIAAPASADRSRAVMS FSAAAVWRIRRAAPLLPTVLLGKTPRYLTSSAATAVGATAVGPSLPALKEYPQLV DRSAAQGRAVYCWNVDEYEDIDFCREVGVAVIGTHHPGRTKAWLEDGRANGTTR
Rv3908	49	VSDGEQAKSRRRRGRRRGRRAATAENHMDAQAGDATPTPATAKRSRSRSPRRG STRMRTVHETSAGGLVIDIGDPRDAQVAALIGRVDRRGRLLWSLPKGHIELGET AEQTAIREVAEETGIRGSVLAALGRIDYWFVTDGRRVHKTVHHYLMRFLGGELSD EDLEVAEVAVVPIRELP SRLAYADERRLAEVADE- LIDKLQSDGPAALPLPPSSPRRRPQTHSRARHADD SAPGQHNGPGPGP

Table 2. DNA sequences of selected low oxygen induced antigens		
Rv no.	Seq ID no.	Sequence
Rv0079	50	gtggaaccgaaacgcagtcgctcgtcgtatgtgcacccgagccatcgcaacgcgcg ggaattcccggatgtcgccgtattctccggcgccgggctaacgcacatcccaggccg aacggttggtcgtcgccgtgggtcgcggttgcccgatcgggcgctcacgggggt gctcgggtgcggctgacatggcgaaactgcgccgatgggcccagcgtggtgcagat aaacctgcaggtaggtgacacccattaaagggcgagggccaccgcgggcacg atgatctgcgacccgcactgatcagactggatcgacagatcgtgcggggcgtcggca cagtgggtgccccggccttgcccggtatcgccccgcggcgattgaccacgcggc cgaggcgctagtcacccgcgcgaaaccggctcgtgctaagggcgcgcaaccccggtgc aggcgattgcccgtatggacgccatggactacgacgtgcatttggttcaccgacgc gagacgggggaggacgctgtggtctatcgggctggaccgtcggggctgcggctggc ccgccagcaccacgtatttccccaggatgggtcacgttgctcgccccagccgggc cgccggtgccgctgattgtgaattcgcgtccgacaccgggttctcacggaggccgc gcggtggaccgggcgcgcgaacatggactgccattcctgtttttcacggaccaggc caccggccgcggccagctgctctactcccgtacgacggcaaacctcgggtgatca ccccgaccggtgacggcgttgccgacggtctggca
	51	atgagcccgggctcgcggcgccagcccgcaaagcggccgggaggtggctcagct cgaccgtgacgaggcgatgcgggttgctggccagcgttgaccatgggcgtgtggtgt tcacccgcgcggcgctgcggcgatccgtccagtcaatcacctcgtggtcgacgggt cgggtgatcgggcccacccgcctgacggccaaggtgtccgttgccgtgcgatcgag cgccgatgccgggtgtcgtggctgcctacgaagccgacgaccttgatccgcggcgtc ggacgggggtggagtgtggtgggtgacgggactggcgaccgaggtcagcgatcccag caggttgcccgtaccagcggctgctacacccgtgggtgaacatggcgatggacac cgtggtcgcgatcgaaccgagatcgtcaccggcatccgcacgttgctgactcgc gtacgccg
Rv0081	52	gtggagtcggaaccgctgtacaagctcaaggcggagttcttcaaaaccttgcgca tccggcgcgatcaggatatttgagctgctggctcagcgggaccgttcggtcgggtg agttgctgtcctcggacgtcggcctggagtgcgcgaacctgtcccagcagctgggt gtgctacgcggggcggtgtgtgcggcacgtcgtgacggcaacgcgatgatcta ttcgattgccgcacccgatatcgccgagctgctggcggtggcacgcaaggtgctgg ccagggtgctcagcgaccgggtggcggtgctagaggacctccgcgcggcgccg- tcggccacg
	53	atgcctatcgcaacgcggaggtctacgcggagatgctcggtcaggccaaacaaaa ctcgtacgctttcccggctatcaactgcacctcctcggaaccgtcaacgcgcgca tcaaaggtttcgcccagcggcgagtgacggaatcatccagttctcgaccgggtggc gcagaattcggctcggcctcggggtcaaagacatggtgaccgggtgcggctgcctt ggcggagttcacccacgttatcgcggccaagtaaccgggtcaacgtggcgctgcaca ccgaccactgccccaaaggacaagttggacagctatgtccggcccttgctggcgatc tcggcgcaacgcgtgagcaaggtggcaatcctttgttccagtcgcacatgtggga cggctcggcagtgccaatcgatgagaacctggccatcgcccaggagctgctcaagg cggcggcgccgccaagatcattctggagatcgagatcggcgtcgtcggcgccgaa gaggacggcggtggcgaacgagatcaacgagaagctgtacaccagccggaggactt cgagaaaaccatcgaggcgctgggcgcgggtgagcacggcaaatacctgctggccg cgacgttcggcaacgtgcatggcgtctacaagcccggcaacgtcaagcttcgcccc gacatccttgcgcaagggaacaggtggcggcggccaagctcggactgccggccga cgccaagccgttcgacttcgtgttcacggcggtcgggttcgcttaagtccggaga tcgaggaggcgctgcgctacggcgtggtgaagatgaacgtcgacaccgacaccag tacggttcacccgcggatcgccggtcacatgttcaccaactacgacggagtgtc caaggctcgatggcgaggtgggtgtcaagaaggtctacgacccgcgcagctacctca agaaggccgaagcttcgatgagccagcgggtcgttcaggcggtgcaatgacctgcac tcgcccggaaagtccttaaccac
Rv0363 C		

Table 2. (continued) DNA sequences of selected low oxygen induced antigens

Rv no.	Seq ID no.	Sequence
<b>Rv0569</b>	54	atgaaggcaaaggtcggggactggctggatcaaaggcgcgacgatagatcaacc ggaccaccgaggggttgattattgaggtgcgctcatccgatgggttcgcccgcggtatg tggtgcgctggctcgagaccgacatgtggcgacgggtgattccgggtccggatgcg gtcgtggtcactgcggaggagcagaatgcggccgacgagcgggcgagcatcggtt cggcgcggttcagtcggcgatcctccatgccagggggaacg
	55	atgggtgagcacgccatcaagcggcacatgcggcaacggaagcctacgaagcatcc cctagcccagaaacggggcgcgcggtattctggtcttcaccgacgatccccgcagga gcgtcctcatagtgcgggttgccacctggattccatgcgcccagaaaaagaacgcg tactacttccaggacggcaatgcgttggttgggatgggtgtctcgggcggcacgggt tgagtacgacgccgacgaccgcacatatgtcgtgcagctcaccgacggaaggcaca ccactgagtcattcttgcgaactcatcgccgagtcgatcacctcaatccgat- gaccta
<b>Rv0572</b> <b>C</b>	56	gtggctggcaatcctgatgtggtgacgggtgctgctgggcggtgacgtcatgctcgg ccgtggcgtcgatcagatcctgcctcatccggcaaacgcgaattgcgcgaacgggt atatgcgggatgcgaccggctatgttcgcctggccgagcgggtgaacgggcgcat ccgtccccgtggattggcgctggccctggggcgaggcgttggcggtccttgagaa caccgcgaccgacgtctgtttgatcaatctggagacgacgatcaccgcgacgggtg aattcgccgaccgcaaaccgggtctgctaccggatgcacccggataacgtgccggcg ctgacggcatttgcggccgcacgtgtgcgcgtggccaacaaccacattctcgattt cggctaccaggggctgaccgatacggctcgcggtctcgccggtgcggggatccaga gtgtcggggcgggagccgatttgcctgcgcgtcgccgctcgccgctagtcacgggt ggccatgaacgcggggtgatcgtcggctcggtagcggcggaatccagcggcgctcc cgaatcctgggcccgcgcgcgacccggcccgagtggttgatccgggatccgg cgcaacgcgacgtcgccgacgatgtggcggcacaggtgctggcggaacaacgcccc ggcgatatcgccatagtcctcgatgcattggggatccaattggggctatgcgaccgc acccggcgacgtcgcttcgcgcaccgactgatcgacgccggcatcgacatgggtcc acggacattcctcgaccatccgcggccaatcgagatatatcgcggtaaaccgatc ctgtacggatgcgggtgacgtcgttgacgactacgaagcactcggtcgccgagtc gttcgcgagtgaactgcgactgctgtatctgaccgtcaccgatccccgccagcggga acctgatctcgtgcagatgcttccactgcgagtgctcgcggtgagcctacagcgt gcctcccagaccgacaccgaatggctccgcaacaccattgagcgcatcagccgccc gttcgggattcgagtcgtgactcgacccgacaacctgctggaggctcgttcccgcgtg ccaacctaacgagcaaggag
	57	gtgacagaccacgtgcgcgaggcggacgacgcgaacatcgacgatctgttgggcca cctgggcggtaccgcgcgcgcgagcgtgcgaagcttgtagtggtgctcgagc agggcatcacccccagcagatctggggcgaccaaccgctgctgctggccacc cgccacctcgtcggcgacgacggcacctacgtatccgcaaggagattagcgagaa ctatggcggttgacctcgagctgctgcagcgggtgcagcgcgctgctcggtctggcca gagtggtgatcctgacgcggtggtgcacatgcgtgcccagcggtagggcgccgca cgcgacagcgggttcgttgagctggggctgaatcccgaaccaagtcgtgctggtcgt gcgtgtgctcgccgagggttgtcacacgccgcccagggccatgcgctacaccgcgc tgaggccattatgcggccgggggtaccgagttggacatcgcaagggtcgag gcgtggtgagccagatcgtgccgctgctggggccgatgatccaggacatgctgtt catgcagctgcggcacatgatggagacggaggccgtcaacgccggagagcgtgcgg ccggcaagccgctaccgggagcgcgacaggtcaccgttgccctcgccgacctggte ggtttcacccagctaggcgaagtgggtgcggccgaagagctagggcacctcgccgg gcggctggccggcctcgcgcgtgacctgaccgctccgcccgtgtggttcattaaga cgatcggcgacgcgggtcatgttgggtctgtcctgatccggcgccattgctggacacc gtgctgaagctggtcgaggtcgctcgacaccgacaacaacttccccgggtcgagc cggcgtcgccctccgggatggcggttagccgggcccggcgactgggtcgccagccgg tcaacgtggcaagccgggtgaccggggtggcgcgcccgggtgccgtgctggtcgcg gattcgggtgcgggaggcccttgggtgatgccccgaagccgacggatttcagtggte cttcgcccggccccgctgcctcaggggaatccgggggtgacgtcaggctttttcgag tccggcgaggggccaactcgacccggctccggcggcggcgcccaagacgacgattt gccggctcgtcaccg
<b>Rv1264</b>		



Table 2. (continued) DNA sequences of selected low oxygen induced antigens		
Rv no.	Seq ID no.	Sequence
Rv1592c	58	atggtagagccccggcaatttggcaggcgcgaccggcgccgaatggatcggccggcc accgcacgaggaattgcagcgcaaaagtgcgcccgtgctgccatccgacgatccgt tctacttcccacctgcccgtaccagcatgcccgtgcccgaacgggtgttgcgctcg cgcatgtcgaactggcgtttatgggcttgattccgcagcccgtcaccgctaccca gctgctgtaccggaccacgaacatgtacggcaacccccgaggcgacgggtgaccacgg tgatcgctcccagcgagcttgccccgggtcagacctgccccttgctgtcgtaaccag tgtgcgatcgatgccatgtcgtcgcgctgtttccgtcatatgccctgcgacgacg ggccaaggccctggggctactgacccaaatggagctgttgatgatcagcgccgcac ttgccgaaggatgggcggtatcagtaccgcacatgaaggggccgaaagggtgtgg gggctgcgctatgaacccggttacccagtgctcgcagcgaatccgggctgccttgaa ttccgagcgtgtcgggttgctccccggcaacgccgatcgggctgtggggctactccg gcggcgggctggccagcgcggtgggcccgcgaagcatgcggcgagtacgcaccggac ctgacatcgctcggcgcgctgctgggatcaccgcgtgaccttggtcacacggtt ccgcggctcaatggcaactcttcttgccggtctgcccgcgttggtggtggccgcgc tgcaacacagctaccccggcctggcccgggtgatcaaggagcacgccaacgacgaa ggacgtcagctgctggagcaactgacggagatgacaacggtagacgcagtgatccg gatggccggcagggacatgggtgacttcctcgcagcaaccccttgaggacattctgt cgacgcgggaaatttcccatgtcttcggcgacaccaagctgggtagcgcgggtgcc accccgcgggtattgatcgctgcaggccgtgcatgactacctcatcgacgtctctga catcgacgcgctcgctgacagctatacagccggcgcccaacgtcacctaccacc gcgacctgttcagcgaacatgtgtccctgcacccgctgtcggccccaatgacgctt cgtgggtcaccgacgggttcgcccggcaagccactgaccgaccaccgcgtccggac cacgtggccgaccatcttcaacccgatgacctacgcccgcagtgccgagactggccg tgatcgccggccaaggtgatcaccggcaggaagttagaccgcgctccgctc
Rv1733c	59	atgatcgccacaaccgcgatcggtgaaggagccaccatgatcacgttttaggctgcg cttgcccgtgcccggacgatactgcgggtgttcagccgcaatccgctgggtgctggga cggatcgactcgaggcggctcgtcatgctgctggccgtcacggtctcgtgctgact atcccgttcgcgcgcgcccggcaccgcagtcacaggttcccgcagccacgtcta tgcccaccaggcccagaccgccatcccgcgaaccgcgaccgtgatcgatcacgagg ggggtgatcgacagcaaacacgaccgccacgtcagcgcccgcgcacgaagatcacc gtgcttgcggatgggtcgtgaacggaatagaacgcagcggtgaggtcaacgcgaa gccgggaaccaaataccggtgaccgcgtcggcatattgggtcgacagtgcgggtcagc tgggtcgatgaaccagctccgcggcccggtgccatttcgggatgcggccctggccgcc ttgggactctggttgagcgtcgcgcgggttcggggcgcctgctggcgctcactcg ggcgattctgatccgcgttcgcaacgccagttggcaacacgacatcgacagcctgt tctgcacgcagcgg
Rv1734c	60	atgaccaacgtcggtgaccagggggttgacgcggtcttcggggtgatctaccacc tcaggctcgcgctggtcagtttcggcaagccggcacaacgagtttgccgctcgacg gcgcatccacgtcatgacgaccgtgctggctacgctgcccgtgaccacgggtgc agcgatgaccatcgccggcgcgctgttcttctgtcgatcaacgagctgacgcggtg cgccgcagtaacagga

Table 2. (continued) DNA sequences of selected low oxygen induced antigens

Rv no.	Seq ID no.	Sequence
Rv1736c	61	gtgacggtgacaccacggaccggcagccgcacgcagagctgcttgacgcagcgg ccggttcttcatcccgggtgagatctcggcggatctgcgtaccgtgacccgcccgcg gcggccgcgacggcgacgtgttctatcgagaccgggtggagccacgacaaggtggtc cgctccacacacggggtgaattgcaccgggtcggtgttcttggaagatctacgtcaa agacgacatcatcacctgggagacgcaggagaccgactatccgtcgggtgggcccgg accggcccagagtatgagccccgcgggtgccgcgcggcgcggttttctctggtac acgtattcgccgacgcgggtgcgccatccgtacgcccgcggtgcttgtcgagat gtatcgggaggcgaaggcacgtttgggtgatccgggtggcgccctgggcccacatcc aggccgaccccgcgggcgccgcgcgcgtaccagcgcgcccgcggaaggcgggctg gtccgggtcagctgggcccaggccaccgagatgatcgccgcgccacgtgcacac catctccacatacggcccggaacgggttgccggcttctcccccatcccggcgatgt ccatggtgagccacgcgcggggtgcgggttcgtggagctaatacggcggggtgatg acgtcgttctacgactggtacgcgcacgtgccgggtggcctccccgcaggtgttcgg cgaccagaccgacgtgccggagtcgggagattggtgggacgtggtgtggcaatgcg cctcgggtgctgctgacctaccgaaactcacggcaactcggcaccgcagaggaattg ctggcccacatcgacggtcgggcccgcggatctgttggggcgcacgggtctctgagct gcgcggtgcgcatccgctgacgcggcgaccgcgtacgtcgacaccttcgacctgc gaggccgcgcaccctgtacctgacctactggaccgcggcgacaccgcgaaccgc ggccgggagatgctggccttcgccagacctaccgcagcaccgacgtcgcaccacc gcgcggcgagacccccggacttctgcgggtggtgctcgaattcgccgcgaccgtcg accccgagggcgggcgacggttgctgagcgggtaccgggtgcccatcgccgcgctg tgcaatgccctgaccgagggcgcattgccatacgcacacacgggtggccgcggtatg ccggacgggtgacatgatgggcgaactcttctggaccgtcgtgccgtatgtgacga tgacgatcgtcgcggtcggctcctggtggcgctaccgctatgacaaattcggctgg accaccgcgtcgtcccagctgtacgagtcgcgggtcgtgcggatcgccagcccgat gtttcatttcggcatcctggtggtcatcgtcggccacggatcgggctcgtgatcc cgcagtcgtggactcaggccgcgggtttgagcgagggcgcatatcacgtgcaggcc gtcgtgctggggtcgatcgccggcatcaccaccttgccggcggttaccctgctgat ctaccggcgggcgaccccggggcgggtgttcattggtaccaccgtcaacgacaagg tgatgtacctcgtgctggtggcgggcgatcgtcgcgggactgggtgcgacggcggtg ggctccggcggttgctcggcgaggcggtacaactaccgcgagacgggtgctgggtggtt ccgctcgggtgtgggtactgcaaccgcgcggggacctgatggccgaggctccgctgt attaccagatccatgtgctgatcgggttggcggttgttcgcgttgtggccgttcacc cggctggtacacgcgttcagcgccccgatcggctatctgttccgcccgtacatcat ctaccgcagccgcgaggagctggtgctaacgcggccgcggcgggcggggtgg

Table 2. (continued) DNA sequences of selected low oxygen induced antigens		
Rv no.	Seq ID no.	Sequence
Rv1737c	62	atgagagggcaagcggccaatctcgtgctggccacctggatctcgggtggtcaactt ctgggctggaacctgatcgccccgctgtcgaccagctacgcgcgtgacatgtcac tgtccagcgcgaggcgtcgtgctcgtcgccaccccgatcctgggtgggtgccett ggccgcatcgtcacccggccgctcacccgaccgcttcggcgggcgcgccatgctcat cgcggtgacgctggcgtcgatcctcccggtgctcgcggtcggggtcgcggaacca tgggtcctacgcgttgcgtggtgttttcgggctcttcctgggcgttgccggcacg atcttcgcgcgtcgccatcccgttcgccaacaactggtaccagccggcgcggcgcg ttctccaccggcgtgttcgggtatgggcatggtcggcaccgcgctctcggcggtct tcaccccgcggtttgtacggtggttcggcctgttcaccacccacgccatcgtcgcg gccgcgtcgcgctcgaccgcggtggtggccatggtcgtgcttcgtgatgcacctta cttcgggccaacgcgcgaccggtgctgccaggctcaaggccgcggcacggttgc cggtgacctgggagatgtcgtttctgtacgcgatcgtgttcggcgggttcgtggcg ttcagcaactacctgccacctacatcaccacgatctacgggttctccacggtcga cgcgggcgctcgaccgcgggttcgccctggcgcggtgctggcccgcccggtgg gcgggtggctctccgaccggatcgacccgaggcacgtggtgctggcctcgctcgcc gggaccgcgctgctggcggttcgcccgcggttgccagccgcgcggagggtgtggtc ggcgccaccttcataccctggcggtctgcctcggcgtgggcacccggcggtgt tcgcgtgggtggcccgccgcgcggcgcccgcatcggtcggtcggtcacccgaatc gtcgccgcggcaggcggtattggcggttacttccgcgcgctggtgatgggcgcgac ctacgaccgggtcgacaacgactacacgggtcggttgcgtgctggtggcgaccg cgctggtcgcgtgtacctacaccgcgctgcacgcgcgggagccggtgagtgaggag gcgtccagg
	63	atgtgcggcgaccagtcggatcacgtgctgcagcactggaccgtcgacatatcgat cgacgaacacgaaggattgactcgggcgaaggcacggctgcgttggcgggaaaagg aattggtgggtgttggcctggcaaggctcaatccggccgaccgcaacgtccccgag atcggcgatgaactctcggtcgcccgagccttgccgacttgggggaagcgaatgtt gaagggtgcgaccacgacatcgaagctgttaccocatcagccggcgcgattgttg- tat

Table 2. (continued) DNA sequences of selected low oxygen induced antigens		
Rv no.	Seq ID no.	Sequence
<b>Rv1739c</b>	<b>64</b>	atgattccacgatgacatcggccggctgggcaccaggggtggtgcagttccgcga ataccaacggcggttggtgctgcgcggcgatgtcctcgccggcctgaccgtggccgcct atctgatcccgcaagcgatggcgatgacgacggtggcgggcctaccgcccgcagcc gggctgtggcgctcgatcgcgccgcttgccatttacgactgctcggatcgctcccg gcagctttcaatcggcccgggaatccgccaccgccttgatgacggcgggcggtgctcg ctccgatggccgcggggatcttcgacgctatgccgttctggcggaaccctcgga ttgctagtcggccttatctgctactcgctggcacggcgcgactaggtttcctcgc cagcctgcatcgcgccgggtgctcgctcggaatacatggccggcatcgcgcttgctca tgatctccagccaactcggcactatcacggcacctcggtcgaaggcaacgaattc ttcagcgaagtacactctttcgcgactagcgctcacgcgagttcactggccgacttt tgtgttagccatgtctgtcctagcgctgctaactatgctcacgcgggtgggcgcgc gcgccccggaccgatcatcgcggttcttgccggccacgatgctagtggccggttatg tccttgatgccaaaggatattcgatttggtgggtcggtacettccggctcccgac gccgggtgtgcgcggcgtttcggtggaagacttgccggcactgatcattccggctg ccgggatcgcgattgttaccttcaccgacgggtgtggtgaccgcacgcgccttcgcc gctcgctcgaggtcaggaagtcaatgccaacgcgcgagctgcgcgcgggtcggggcctg caacatcgccgcgggctgacacacgggttttcgggtgagttccagcagcagccgta ccgccctcgccgacgtcgctcggtggccgcacccagctgtactcgctgatcgcggtg gggcttggtgtcatcgctgatgggttttcgcgagtggtgctgctggccatgtttccgat cgccgctctgggcgctttgggtggtatgcccgcgctacgcttgatcgacttgctcag aattccggcgactggcgcggtttcggcgcagcgaactcatgctggcactagccacc acagcagccgtgttaggcctaggagtggtctatggagtcctcgccgcgggttgccct gtccatcctcgaactgcttcgctcggttcgcacatccgatgacagcggttctcggtg tcgtgcccggcattgcccggcatgcacgacatcgatgactatccgcaggccaaagcgc gtgcccgggctgggtggtgtatcgctatgacgcgcgcttggtgcttcgccaatgccga agacttcgcgaggcgagcactgaccgtggtcgatcaggatccggggcaagtcgagt ggttcgtactcaacgcgcgaatccaatgtggaggtcgacctgactgcgctggatgcg ctcgaccaactccgcaccgagctgctgcgtcggggaatagtggttcgccatggcccg ggtcaaacaagacttgctgaatcactcagggccgcagctcttctcgataagattg gcgaagaccatatctttatgacattgcctaccgcagtgaggcggttcgctcggcgc
<b>Rv1813c</b>	<b>65</b>	atgatcacaacacctccgacgcgcgaaccgcgatggcagccgcggcctaggggctgc tctcgggctgggcatectgctgggtcccgacggtggacgcccattctcgccaacggtt cgatgtcggaagtcgatgtcggaattgccgggttgccatatccctccgattatc cattacggggcgattgcctatgccccagcggcgcgctcgggcaaaagcgtggcacca gcgcacaccggcgcgagcagagcaagtcgactagaaaagtcggtgacaagactt gcaaagtgggttagtcgcttcaccaggtgcggcgcggtcgccataaacggctcgaaa taccaaggcggaaccggactcacgcgcgcgcggcgagaagacgacgcccgtgaaccg actcgaaggcgggaggatcgtaactgggcgtgcaac

Table 2. (continued) DNA sequences of selected low oxygen induced antigens

Rv no.	Seq ID no.	Sequence
Rv1997	66	ttgtcggcgctcagtgctctgccacgacggctcatcatggcttgccagcacatgaagt ggtgctgctgctggagagcgatccatatcacgggctgtccgacggcgaggccgccc aacgactagaacgcttcgggcccacacaccttgccgggtggtaacgcgcgctagcttg ctggccccgcatcctgcggcagtttcatcacccgctgatctacgtttctgctcgttgc cgggacgatcacccgctgtcttaaggaattcgttgacgcgcgagtgatcttcggtg tgggtggtgatcaatgcgatcgtgggtttcattcaagaatccaaggcagaggccgca ctgcagggcctgcgctccatgggtgcacacccacgccaagggtggtgcgcgagggtca cgagcacacaatgccatccgaagagctggttcccgggtgaccttgtgctgttagcgg ccggtgacaagggttcccgcgatttgcggctggtgcgacagaccggattgagcgtg aacgagtcagcacttacccggcgagtcgacgcgggttcacaaggacgaggtggcgtt gccggagggcacacccggtcgtgatcgtcgcaatatcgctattccggcacattgg taaccgcggggccatggcgccgggatcgtcgtcgacccggcgccgaaaccgaactc ggtgagattcatcggctcgttggggccgcgaggttgctgccacacccgctgaccgc gaagctggcgtggttcagcaagtttctgacctcgccatcctgggtctggcagcgc tcacgttcggcgtgggtttgctgcgcgggcaagatgccgtcgaaacgttcaccgct gcgatcgcgctggcgggtcggggcaattcccgaaggctcgcacccgcccgtgacct caccttggccatcgccatggcccggtatggcgaagcgccgcggtcattcgacgtc taccgcgggtgaaacgctgggcagcaccacggtcatctgcgcgccaagaccgga acgctgaccgagaatcagatgacggtccagtcgatctggacacccacggtgagat ccgggcgacccggaacgggctatgcacccgacgtcctcctgtgcgacaccgacgacg cgccggttcgggtgaatgccaatgcggcccttcgctggctcgtgctggccggtgcc tgcagcaacgacgcgcgactggttcgcgacggcacacgctggcagatcgtcggcga tcccaccgagggcgcgatgctcgtcgtggccgccaaggccgggttcaaccgggagc ggctggcgacaactctgcgcgaagtggcagccataccggttcagttccgagcggcaa tacatggccacctgcatcgcgacgggacggatcatgtggtgctggccaagggtgc tgtggagcgcatgctcgacctgtgcggcaccgagatgggcgcccagcgcgcatgtgc ggccgctggaccgcgccaccgtgttgctgcccaccgaaatgttgacttcccggggg ttgcgggtgctggcaaccgggatgggtgccggcgccggcactcccagcacttcga cgaaaacgtgataccaggttcgctggcgctgaccggcctgcaagcgatgagcgatc caccacgagcggccgcggcatcggcgggtggcgccctgccacagtgcgggcattgcg gtaaaaatgattaccggtgaccacgcgggcaccgcccaggtcgcaaccgaggt ggggttgcctcgacaacactgaaccggcggcaggctcgggtcctgacgggtgcgagc tggccgcgctgagcgcgagaccagtaccggaggccggtggatcacagccagcgtgtt gccagggtctctcccagcagaagctgcggttggtgcaagcattgcaggccagggg gcacgtcgtcgcgatgacggcgacggcgtcaacgacgccccggccttgcgtcagg ccaacattggcgtcgcgatggggccggtggcaccgaggtcgccaaggatgccggcc gacatggtgttgaccgacgacgacttcgccaccatcgaaagccggtcgaggaagg ccgcggtgatttcgacaatctgaccaagttcatcacctggacgtgcccaccaacc tcggtgagggcctagtgatcttggccgcatcgtgttggcgtcgccttgcgatt ctgcccacccaaattctgtggatcaacatgaccacagcgatcgcgctcggactcat gctcgcgttcgagcccaaggaggccggaatcatgaccggccaccgcgacccccg accaaccgctgctgacggctggttgtcaggcggactcttctggtttccaccttg ctgctcgcagcgcgtggtggctgttgcacatgggagctcgacaatggcgcgggcct gcatgaggcgcgacggcggtcgtgaacctgttcgtcgtcgtcgaggcgttctatc tggtcagctgccggtcgtgacccgatcgccctggcggtcggcatgttcgccaac cgctggatcatcctcggcgtcagtcgcgaggccatcgcgcaattcgcgatcacata tctaccgcgatgaatatggtgttcgacaccgcgccaatcgatatcgggggtgtggg tgcgcatattcgtgtcgcgaccgcaatcacgattgtggtggccaccgacacgctg ctgccgagaatacgggcgaaccgcca

Table 2. (continued) DNA sequences of selected low oxygen induced antigens		
Rv no.	Seq ID no.	Sequence
Rv1998c	67	atgagtttccacgatcttcatcaccaaggtgttccgttcgtgttgcccaacgcctg ggatgtgccgtcgccctggcctacctcgcggagggcttcacggctatcggcacaa ccagtttcgggggtctcgtccagcggcgggcacccggacgggcaccgcgccactcgc ggcgccaacatcgcaactggcgccgcccctggcaccgctgcaatgctacgtcagcgt cgacatcgaggacggatacagcgacgaacccgacgccattgctgactacgtcgac aactgtcgacagccggaatcaatatcgaggacagtagcgccgaaaagctcatcgac cccgccctggcagccgctaaaaatcgttgcgatcaaacacgtaaccccgagggtgtt cgtcaacgcccgcgtcgacacctattggttgcgcagcagccgataaccaccagca cgatccagcgcgcacttcgctacgtcgatgccggcgccgacggcgctctttgtccca ctggccaacgatcccgcgaacttgctgagctcactcgcaacattccgtgcccgggt taacacggttgccggtgcccggcttgacgatcgccgaccttggtgagctcggggtgg cccggtgtcaaccgggtcagtgccctacagcgccgggttggtatgcagcgccac gcggtcggggcgtgagcgacggagagcagctgccacgggtccgtaccgtacgccga actgcaggcacgcttggttgactacgagaaccgcacgagtacaacg
	68	gtggtcaagcgtctcgggcaaccgactttcgccgagcatctgggtccggatggga atcacctcagtgctcggtccattcgggcgcgattgctgctaccccggggtcggtcgc ggccgcccgaacgccgattgttgctggaatcagctcgcggtgacgcctgacaccggg atgccggcatcgtcgcccgccggcgcgacgcggcgccctacgacgcctggatga ctcaccacccggcgcccgatcctggcgaccgaggtcgccgcttgccgcccgtca tcgaggtctttgccagccacgcttggaatcggtgctcggtaacaggacgtttcgcc gacctgctcgccggtcggttcggactcgatccatcccgtgatgcgtgatgttcgc acgcggcgccggcgtcctggctcgcaatgccgtcgccgagggcggtccctttcgta gccggcacttcggggcggtcctcatggcattcacgctctgtttcgtaaccgaccg gccgccatattccgggaaacgcggcgtctgctcgccgacggcgccgcccctgttat cgggttcttgctcgcgggacaccgtgggcccgaactgtacgctctgcgcgccc gcgacacccaggctaccgcgacgcccgttctacaccgcccgaactcgaacaa ctgctcgcaactcgggattccgggtcatcgcccgccgctgcagctgcacaaacc gccgggactcgcccggtacgacatcgaagccgcccatgacgggtatccaagccggcg ccggcttcgttgctatctcggcggtcgaccaagcgacgagcctaaggatgatcac ccactcgagtcggaa
Rv2005c	69	atgtctaaaccccgcaagcagcagcaggtgtgctcggtcggttagatggttcgctcga atcggtatgccgcgcctgttgggggtgccaccgatgcgcgatgaggaacattccgc tgaccgtggtccacgtggtgaacgccgatgtagcgacgtggccgcccgatgccgtat ccggagacctgggggggttggcaggaggacgagggctcgccagatcgtcgccaaacgc cgtcaagctcgccaaagaggcggttgagcggtatcgaaagctcagcgtaaagagcg agctcgtattttccacgcgggtacctaccatggttgaaatctccaacgaggcagag atggtggtgttgggcagctcgggcggggagcgctggcccagaggcttgctcggttc ggtcagctcgagcctggtgcgacgcgcgggtgcccggtcgcggtcatccacagcg atgatgcgggtgatccctgatccgcagcagctcccgtgctggtgggaatcgacgggt tcgcccgtttcgagcttgcgacggcggtggcatttgacgagggctcgcccgcg cgtcgaaactgatcgccgtgcacgcgtggagtgacgtcgaagtgggtggaactccgg gtttggacttctcggtgtacagcaggaagcgagcttagtctcgccgaacgcttg gcaggttggcaagaacgctatcccgatgtgccgggtgagccgggttgctcgtttgcga tcgcccggcgcggaagctggtgcaaaagtcggcggtccgcccagcttgctcgtcttg gcagtcattggccgaggtggcttgaccggcatgcttctggggtcggtcagtaacgcg gtcttacacgcgcgcgggtgccagtgatcgtggcacggcagtcg

Table 2. (continued) DNA sequences of selected low oxygen induced antigens		
Rv no.	Seq ID no.	Sequence
Rv2007c	70	gtgacctatgtgatcggtagtgagtgcgtagtgatggacaagtcctgtgtgca ggagtgtccggtcgactgtatctatgagggcgccgaatgctctacatcaaccccg acgagtgcgtggattgtggtgcgtgcaaaccggcctgccgcgtcgaggcgatctac tgggaaggcgatctaccgcagatcaacaccagcatctgggggacaacgcgcctt ttccaccaagtccctgccgggcccagtggtcgctccgctgggttcgccgggtggcgccg cagcgggtgggcccgatcggagtcgacacgcctctggtcgcggtatcccggtg- gagtgcct
	71	atgaaccaatcacacaaacccccatcgatcgctcgctcggtattgatggctcgaagcc ggccgtgcaagccgcactgtgggcggtcgacgaggcagccagccgtgacatcccg tgctgtctgctgtacgcgatcgaacccgacgatcccggtacgccgcacacggcg gcccgtcgcaaactcgccgcgcgcgcgagaacgcggtgcgctacgcgttcacagcgg cgaggcgccgacggcgccggtcaagtcgaggtggagatcacccaggagcggccg tcacctcggtgatccgcgcttcggcggtgctgcccgtggtgctggtggcgctatc ggcgtgcaccacttccgaccggagcgggtgggatctaccgcagcggccctggcggt atcgccgcagtgcccagtgccgatcgctgcgacccccaccgggtccccatcggaacgc acgccgatggatcgctcgctcgaggcggaacgggtcgctccgatcgggtgtttgctg ggggcggtgatggccgaagcacggctgcgcgactcgccggttcgggtggctcacctg ccggcaatccggagtgggcgataccggggacgacgtccgtgccagcctggaccgct ggcttggccgttggcaaccacggtatcccgatgtgcggtgcaatcgccggcagtg cacggcgagctgctggattatctggctgggtcgatcggtacacatgggtggt gctcagcgcgagcgaccaggagcatgtggagcaacttgtgggagcgccgggcaacg ccgtgttgccaggaggccggctgcacctgctggtcgctcggtcagcagtatctg
Rv2029c	72	atgacggagccagcggcggtgggacgaaggcaagccgcgaatcatcactttgaccat gaaccccgcccttggacatcacgacgagcgtcgacgtggtgcgcccgaccgagaaaa tgctgttggtggcgacctcgctacgatcccgggcgccggcggtatcaatgtcgccgc attgtgcatgtcctcgccggttgctcgacagcactgttcccggccggcggtcgac cgggagcctgctgatggcgctgctcggtgatgcgggagtgccatttcgctcattc cgatcgccgctcgacgcgggagagcttcacggtcaacgagtcaggaccgccaag cagtatcgtttcgtgcttcggggccgctcgctgaccgtcgcgagcaggagcaatg cctcgacgaactgcgcgggtgcggcggttcggccgcttctggtggccagtgga gctgcccgcagggtgtggctgccgactactatcagcgggttgccgacatctgccgc cgatcgagcactccgctgatcctggatacatctggtggcggttgccagcacatttc gtccggggtgtttcttctcaaggcgagcgtgcgggaactgcgcgagtcgctcgat ccgaactgctgaccgagcccgaacaactggccgcccacacgaactcattgaccgt ggcgcgccgagggtcggtggtgctcgtctggatctcagggcgcgctattggccac acgacatgcgagccatcgattttcgtcgattccgatgaccgcggttagcggtgtcg gcgcggcgacgcgatgggtggcgcgattaccgtggccctcagccgtggctggtcg ctcatcaagtccgttcgcttgggaaacgcggcaggtgcagccatgctgctgacgcc aggcaccgcggcctgcaatcgcgacgatgtggagaggttcttcgagctggcgccg aaccaccgaagtccggcaggatcaatacgtttggcaccgcgatcgtaaccgcgaa gcctcgcca

Table 2. (continued) DNA sequences of selected low oxygen induced antigens		
Rv no.	Seq ID no.	Sequence
Rv2030c	73	gtgctgatgaccgcagcggtgatgtcaccgcgctcgccgcggcgctgttccg tgaccgcccgcgaggccggccgggtgctggcggaattactcgccgcctatcgggaac agccggacgtgattgtgctcggttggcccgggtggcctcccgggtcgcatgggag gttgcccgcgactgcatgccccgctagacgccttcgctcgcaaaacttggtgc cccggggcatgacgagttcgccgttgggtgcaactggccagcgccggcgctggg tcaatgacgacgtcggtcggggctcgggatcacaccgcagcaactgcgcgacatc gccgaacgtgagggtcgggaactgcttcggcgcgagtcgcctaccgcggcgagcg cccgcccaccgatataccggcaagacgggtcattgtcgctcgatgacggtttggcca ccggcgcaagcatgttcgcgcggtacaggcattgcgcgatgcgcaaccagcgag atcgtgattgccgtgcccgcggcgccggagtcacgtgcccgggagttcgccggcct cgtcgacgacgttgtgtgcgcgaccatgccgaccccgcttccctggccgctcggtgag cgttttgggacttcggcgaggtcaccgacgaggaggtccgcccgtcctggccacc ccgaccgctgggcccgtcgctgcccggcccgcggtcaaccggcgccgatgttct gcgcagagtcgcatcgacgccccgggggtgttcgacgcacgaggtgttggcgg agctggtcggcgatgcacgaatcgtgttgatcggcgaaagctcgacggcacacac gaggttctaccaggcccgggccgcatgacacagtggtgatcgaggagaagggtt tgggtcggttagccgcgaggcgactggcccgcgcctaccgggtcaatcggtacg ttcgcgccctcggcgaggacaccaacgctgacgaggcgcttagcggattcgagcgg tttcccgcctggatgtggcgcaaacccgtggtccgagattttgtggaatggctgcg cacacgcaaccagcgctacgagtcgggcgcgctgcggaagccggcttctacggtc tggatctttacagcctgcatcggtcgatccaagaggtgatcagctatctcgacaag gtcgaccccgctgcgccgacggcgcgccggccggtatgcgtgcttcgaccatgc ctgcgccgatgacggtcaggcgtacggattcgcgcccgcatcgggcgcgggtccgt cgtgcgaacgtgaagccgtcgagcaactggtcgacgttcagcgcaatgccctggcg tatgcgcgccaagacgggctgcttgccgaggacgaactgttctacgcccagcaaaa cgcgagacggtgcgcgacgagaggtgtattaccgggcatgttcagtggaacg ttacctcggtggaacctgcgcgaccagcacatggcgagacccttggcagtttgctg acgcattttgaccgacacctcgatgcgcgccggcgcgcaatagtggtgtgggctca taactcccacgtgggtgacgcacgcgctaccgaggtgtgggcccagcggcagctca ccctcgccagatagtcggtgagcgatacggtagcaggtcgcgagcatcggtatc agcacgtacacgggacacgtcaccgcggccagcgaatgggggtggtatcgcccaacg caaagcggttcgccggcactgcacggcagtgctcgaggagctctccaccagactg cagacagtttcctggtgtcagcgcggttaagccgcgacgcccgaagccccgctggac gttgtccggttgggacgtgccatcggcgtcgtttatctaccggcaacggaaaggca aagtcactacttgacgtgcggcccgccgaccagttcgacgcatgatccacatcg atcagaccggtgccctggaacctctcgaggtgacgagccggtggatcgccggcgag aaccggaaacctaccgacgggtctg
	74	atggccaccaccttcccgttcagcgccaccgcgggtccctcttcccgcagttttc tgagctgttcgcgcccttcccgtcattcgccggactccggcccaccttcgacaccc ggttgatgcggctggaagacgagatgaaagagggcgctacgaggtacgcgcggag ctcccggggtcgaccccgaagacgctcgacattatgggtccgcgatgggtcagct gaccatcaaggccgagcgcaccgagcagaaggacttcgacggtcgctcggaattcg cgtacggttccttcgttcgcacgggtgtcgctgccggtaggtgctgacgaggacgac attaaggccacctacgacaaggcattcttactgtgtcggtggcggtttcggaagg gaagccaaccgaaaagcacattcagatccggtccaccaac



Table 2. (continued) DNA sequences of selected low oxygen induced antigens		
Rv no.	Seq ID no.	Sequence
Rv2032	75	atgccggacaccatggtgaccaccgatgtcatcaagagcgcggtgcagttggcctg ccgcgcaccgtcgtccacaacagccagccctggcgctggatagccgaggaccaca cggttgcgtgttctctgacaaggatcgggtgctttacgcgaccgaccactccggc cgggaagcgtgctggggtgcgcgccgtactcgaccactttcgggtggcgatggc ggccgcgggtaccaccgccaatgtggaacgggtttcccaaccccaacgatcctttgc atctggcgtcaattgacttcagcccgccgatttcgtcacccgagggccaccgtcta agggcggatgcatcctactgcccgtaccgaccggctgcctttcgcgagccgccc ggattgggacttgggtggagtgcgagttgcgcacgaccgtcaccgcccacacgggtgc gcatcgacgtcatcgccgacgatatgcgtcccgaactggcggcgccgtccaaactc accgaatcgctgcccgtctacgatttcgtcgtatcatgccgaactccttttgggtggac aggggcttttgagacttctgaggcataccgcacagttcattgggtatcggcgccg aaagtgaccgggtcaccttcggacgcgacttcccggctcgtcgccaacaccgatagg cgcccggagtttggccacgaccgtctaaaggtcctgggtgctctccacctacgacaa cgaacgcgccagcctactgcgctgcggcgagatgctttcgcgcgtattgcttgacg ccaccatggctgggcttgccacctgcacgtgacccacatcaccgaactgcacgcc agccgagacctggtgcagcgtgattgggcagcccgaactccgcaagccttgggt tcgctcggctcgtggccccggagatggaagagccgccaccggcaacgcctcggcgac caatcgatgaagtgtttcacgttcgggctaaggatcaccgg
	76	atgccactgctaaccattggcgatcaattccccgcctaccagctcacgcgtctcat cggcggtgacctgtccaaggctcgacgccaagcagcccgcgactacttcaaccacta tcaccagtgcgaacacccaggcaagtggcggtggtgttcttttggccgaaagac ttcacgttcgtgtgcctaccgagatcgcgcggttcagcaagctcaatgacgagtt cgaggaccgcgacgccagatcctgggggttcgattgacagcgaattcgcgcatt tccagtggcgtgcacagcacaacgacctcaaaacgttacccctcccgatgctctcc gacatcaagcgcgaactcagccaagccgcaggtgtcctcaacgccgacgggtgtggc cgaccgcgtgacctttatcgtcgacccaacaacgagatccagttcgtctcggcca ccgcccgttcgggtgggacgcaacgtcgatgaggtactgcgagttgctcgacgccctc cagtcgacgagctgtgcgcatgcaactggcgcaaggcgacccgacgctagacgc tggcgaactcctcaaggcttcggcc
Rv2623	77	atgtcatcggggcaattcatctctggaattatcgtcgggatcgacgattcaccggc cgcacaggttgcggtgcggtgggcagctcgggatgcggagttgcgaaaaatccctc tgacgctcgtgcacgcggtgtcgccggaagtagccacctggttgagggtgccactg ccgcggggcgtgctgcgatggcagcaggatcacggggcgccacctgatcgacgacgc actcaagggtggttgaacaggcttcgctgcgcgtggtccccccacgggtccacagtg aaatcgttccggcgccagccggttccacattggtcgacatgtccaaagacgcagtg ctgatggtcgtgggtgtctcggaagtggcggtggccggggccggtgctcggttc ggtcagttccggcctgctccgccacgcgcactgtccggtcgtgatcatccacgacg aagattcgggtgatgccgcatccccagcaagcgccggtgctagtgtgctgtgacggc tcgtcggcctccgagctggcgaccgcaatcgattcgacgaagcgtcgcggcgaaa cgtggacctggtggcgctgcacgcatggagcgacgtcgatgtgtcggagtgcccg gaatcgattggccggcaactcagtcgatggccgagcaggtgctggccgagcgggtg gcgggttggcaggagcggatcccaacgtagccataaaccgcgtggtggtgcgcga tcagccggccccgcagctcgtccaacgctccgaggaagcccagctggtcgtggtcg gcagccggggccgcggcggtacgccggaatgctggtgggtcggtaggcgaaacc gttgctcagctggcgcgagcgcgggtcatcgtggcacgcgagtcgctgact

Table 2. (continued) DNA sequences of selected low oxygen induced antigens		
Rv no.	Seq ID no.	Sequence
Rv2624c	78	atgtctgggagaggagagccgacgatgaaaacaatcattgttggtatcgatgggtc gcacgcggcgattacggccgcattgtggggggttgacgaggccatcagccgagcgg tgccgctgcgactgggtctcagtgatcaagccgacacatccgtccccggacgactac gaccgcgaccttcgcatgctgaaagatcgcttcgggaagcgcagtcgctgttga ggccgcgggcaagctcgtcaagatcgaaaccgacatcccccgcgggccagccggcc cgggtgcttgtggaggcatcgcgcgacgcccagatgatctgcgtcggctccgtggga atcgggcgctacgccagctcgatcttgggttcgacggcaaccgagctggccgaaaa ggcgcatgtcccggtcgccgctcatgcgctcaaaagtggaccagccagcgtctgaca tcaactggatcgtggtgcgcatgaccgacgcaccggataacgaggccgtgctggaa tacgctgcccgggaagcgaagtgtcggaagcgccatactggcactcggcggggcg accggaggagctccgggagattccggacggcgaattcgaaactcgcgtgcaggatt ggcaccacgctcatcccgatgtgcgctctaccgatcaccactcacacgggtatt gcccgggttcctggccgaccacgacgagcgcgtacagctggcagtgatcggcgggtgg tgaggccggtcagctagcgcggctggtcgggccatccggacatccgggtgttcgctc acgcgagtggttcgggtgcttgcgttcgcccgc
	79	atgcgtgatgcgatcccgcgttggggcgatcgccgggtttgtggtgaacgtccactg gagcgtgttggtgatcctgtggttgttcaactggagctctggcgaccatgttgccgg gtaccgtcggaggctacccggccgtggtctattggcttctcggcgacaggtggcgcg gtcatgttgctggcgctcgctgttggctcatgagctcgcgcacgccgtcgctcgctc tcgcgcgggggtatccggttgagagcgtgacgttgtggctgttcggcggggtgaccg cgcttggcgggcgaggcaaaagacgcccgaagccgcttccggatcgcggttcgggggt ccggctaccagcctggcgctgtcggcgacattcggtgcttggccatcacgctcgc cgcgctgcggacccccggccatcgatgacgcttgcgttgggtggttggctactgtca acctgctgctggggctgttcaatctgctgctggcgcgccgttggacgggtggggcg ttgggtccgggcctatctgtggcgccgccacggcgatagtgctgcgcgcgggatcgg tgccggcggggcccggacgggtggttgcgctggtccttgatcgcggttgggattggccg agtttggtgggtggtggcctcgtcggtgggtcgtggttagccttcattggctggttt atcttcgctgcgcgtcgcgaggaggagaccggatttcgacccagcagctgtttgc cggggtgcgtgtggccgatgcgatgaccgcccgaaccgcatacggctcccggatgga tcaatgtcgaggatttcacccagcgttacgtgcttgggtgaacggcactcggcatat ccggttgccgatcgggacggatcgatcacgggcctggtggcattgcggcagctgcg cgatgttgcgcctagccggcgacgactaccagcgtaggtgacattgcgctgcgc tgacacagcgtgccgacccgcccaccacaagagccgctgaccgcgtcctagagcgg atggcacccgctcgcccgcgacgcgtgcgtggtcaccgaaggagcgcggtggt cgcatcgctactcccagcgatgtcgcgcggtgattgacgtctaccggttggccc agccggaaccgacctttaccacgagtccccaagatgcggacaggttttccgatg- cgggg
Rv2626c	80	atgaccaccgcacgcgacatcatgaacgcaggtgtgacctgtgttggcgaacacga gacgctaaccgctgccgctcaatacatgcgtgagcacgacatcggcgcggttgccga tctgcggggacgacgaccggctgcacggcatgctcaccgacccgcgacattgtgatc aaaggcctggctgcgggcctagaccgaataaccgccacggctggcgagttggccc ggacagcatctactacgtcgatgcgaacgcaagcatccaggagatgctcaacgtca tggaagaacatcaggtccgccgtgttcgggtcatctcagagcaccgcttggtcgga atcgtaaccgaagccgacatcgcccgacacctgcccagacgacccattgtgcagtt cgtaaggcaatctgctcgcccatggccctcgccagc

Table 2. (continued) DNA sequences of selected low oxygen induced antigens		
Rv no.	Seq ID no.	Sequence
Rv2627c	81	atggcaagttctgagcgacggcaccacgaacgctcggcttttcgctgagtc accggtcttgagcggcgccatgggaccgttcatgcacaccggtctgtacgtcgtc aatcgtggcgcgactatctgggtcaacagcccagataaactgcccagtcgcacggccc actattgcccttagcggcgcaagcctttcgagacgaaatcgctcgtggtggcctcaa ggcacgacgtccggtcagcaatcatcgagtgttcgagcgcatcagccaagaagtgg ccgctggactggagtctatgggaatcgcatggtcggagaagcctagcggattt tttggccagccccaccgctcaccgaggtcgcgggtccgaaaggtcaaggaccgcag acgctccttttatcgcatcttcttcgacagtggttttacgcgcgcatccgggtgaac cgggcagccaacggtggctctcatacactgcgaacaatcgcgagtacgccctgta ctgcggcaccagagccgctccctggctggtttgtgtacacggcaccgagatggg cagggccccggttgatctcgcggtgttcgcgcgctggaagctgcagacgaactcg gctgaacattgtcatgccggttcttcgatgcaggtccccgcgggcaaggtctg ccgaagggcgccgtttttcccgagagaagatgttctcgacgatgtgcagggacggc tcaagcgggtgtgggatatccggcggtgtgttgcctggatcagatcgaggaggagg agtcgctgatcgggttgaacggtctctcgctgggcgggtacatcgctcattgggtc gccagcctcgaagaaggtctcgctgcgcgattctcggtgtcccagtggtgatct gatcgagtgttgggcccactgcggtcttcggcacaagacccccgcggccaca ccgtcaagatggccgaaccgatcggccgaatgatctcgccgtctcacttacgcca ctggtgcccatgccgggcccgtttatctacgcgggcatcgcgaccgactcgtgca tccacgcgaacaggtgactcgctctgggagcactggggcaaaccgaaatcgtgt ggtatccaggcggtcacactggcttcttcagtcgcggccggtacgacggtttgtc caggtgcgctggagcagtcgggcctgttgacgcgccacggacacagcgcgac- cgttccgcc
	82	atgtccacgcaacgaccgaggcactccggtattcgggctgttggccctacgcag ggccggccgatgtggtcggataggcaggtggggggtgcaccaggaggcgatgatga atctagcgatatggcaccgcgcaaggtgcaatccgcaccatctatcaggtgacc gatcgctcgacgacggcgacagcagcgggtgcctggtagcagatcactagcac cgtgtccggttggtgtcggagttgggcacccaaagccggttggccgatgagcttg cgctgcggtgcggatcggcgactggcccgctgcgtacgcaatcggtagcacctg tccggtgagattgccgttgcggtc
Rv2629	83	atgcgatcagaacgtctccggtggctggtagccgcagaaggtccggttcgctcgg gtatttcgacgactcgcacgacactcttgatgcgctcgagcgccgggaagcgacgt ggcgcgatgtccggaagcatctcgaaagccgcgacgcgaagcaggagctcatcgac agcctcgaagaggcgggtgcgggattctcgaccggccgctcgccagcgtggccgcgc gctgatcgcgaccggcgagcaagtaactggtcaacgagcatctgatcggccaccac cggctacggtgattcggctgtcggattatccgtacgtcgtgccattgatagacctt gagatgcggcgaccgacgtatgtatttgccgcggttgatcacaccggcgccgacgt caagctgtatcagggggccaccatcagttccacgaaatcgatgggggtcgggtacc cgggtgcacaagccggtcaccgcgggtggaacgggtacggcgactccagcacacc accgaagaagccatccgaatgaactgccgcggtgcggcaccatctcaccgact ggtagacgctgccgaccccgaggtggtgttcgtgtccggcgaggtgcgggtcacgca cagacctgctttccacattgccgcagcgggtggcggtccgggtgtcgagctgcat gccggaccgcgcaaaagcgccttagacgaggaagagatctgggacctgacatccgc ggagttcaccggcgggcggtacgccgaaatcaccaatgtcgcacacaatttgagg cggagatcggacgcggatcggggctggcgcccaagggttggcgagggtgtgtgcg gctctgcgtgacggcgacgtcgacacgctgatcgtcggagagctaggcgaggccac cgtggtcaccggtaaagcgcgtactacggtcgcgcgggatgccgacatgttgtccg aactcggcgaaccggtagatcgctggcaaggccgatgaggcgttgccattcgcc gcgatcgcggtaggtgcgcattggtccgtgacgacaaccgatcgcgccactaga tgggggtgggcgcatctgtgcgttatgccgccaccaaccgactcggcagccata- gatcc

Table 2. (continued) DNA sequences of selected low oxygen induced antigens		
Rv no.	Seq ID no.	Sequence
Rv2630	84	atgctgcaccgcgacgatcacatcaatccgccgcggccccgcgggttgatgttcc ttgcgccccgcctacgagcgacaaatcccctgcgcgccttggcgcgttgcttcagg cgggcaagccgggaccagttcagggcatcgggtccgtgcccatacggcggacttg cgaatcgaagcctgggcaccgacccgtgacggctgtatccggcaggcgggtgctggg taccgtcgagagcttccctgacctggaatccgcgcacgcgggtccatacccggtgc gccggctgaccgcggatcgcgacgacgatctactggctgcgggtgctcgaggaggtc atatttttgcgtggacaccgtcggtgaaacgcctgtcgatctcaggctgcgcgacgt tgacgggggtgctgacgtcacattcgcaacgaccgatgcgagtacgctagttcagg tgggtgcggtgccgaaggcgggtgtcactcaacgaacttcggttctcgcagggtcgc cacggctggcgatgtgcggtaacgctcgtatgtg
	85	gtgacgcaaaccggcaagcgtcagagacgcaaattcggctcgcatccgacagttcaa ctccggcgcgtggcaagccagctacaccggccccgacggcgcggtgtacatcgccc ccaaaaccttcaacgcgaagatcgacgcggaagcatggctcaccgaccgcgcgcgc gaaatcgaccgacaactatgggtccccggcatcgggtcaggaagaccgccccggagc cccatcgggtgagtacgcggaaggatgggtgaagcagcgtggaatcaaggaccgca cccgcgccactatcgcaaacgtgtggacaaccacatcctggccaccttcgctgac accgacctacgcgacatcaccccgccgcgctgcgcgcgtggtagccaccaccgc cgtgggcacaccgaccatgcgggcacactcctacagcttgcgtgcgcgcaatcatgc agaccgccttggcgacgacctgatcgactccaaccctgcgcgcatcaggcgcg tccaccgccccgcgctccacaagatcaggccccaccctcgacgagctggaaac catcaccaaagccatgcccgacccctaccaggcgttctgtgctgatggcggcatggc tggccatgcgctacggcgagctgaccgaattacgcgcgaagacatcgacctgcac ggcgaggttgcgcggtgctggcggtgctgtcgttcgggtggcggaaggcttaagg gacgacaccgaaaagcgatgcgggagtgcgcgacataagtatcccgccacatctga taccgcgatcgaagaccaccttcacaaacacgtcaaccccgccgggagtccttg ctgttcccatcggtcaacgaccccaaccgtcacctaccctcggtcgctgacccg catgttctacaaggccgaaagccgcggccgaccagacttacgggtgcacgacc ttcgacactccggcgccgtgttggtgcatccaccggcgccacactggccgaactg atgcagcggctaggacacagcacagccggcgccgactccgctaccagcacgcgcg caaggccgggaccgcgaaatcgccgactgttaagcaaactggccgagaaccag- gagatg
Rv2780	86	atgcgcgtcggtattccgaccgagaccaaaaacaacgaattccgggtggccatcac cccggccggcgtcgcggaactaaccgctcgtggccatgaggtgctcatccaggcag gtgcccggagagggctcggctatcacccgacgcggtttcaaggcggcaggcgcgcaa ctgggtcggcaccgcgaccaggtgtgggcccgcgctgatttattgctcaagggtcaa agaaccgatagcggcggaatacggccgcctgcgacacgggcagatcttgttcacgt tcttgcatttggccgcgtcacgtgcttgaccgatgcgttgttggttccggcacc acgtcaattgcctacgagaccgtccagaccgcgacggcgactaccctgcttgc cccgatgagcgaagtgcgcggctcgactcgccgccaggttggcgttaccacctga tgcgaaaccaagggggccgcggtgtgctgatggcggggtgcccggcgtcgaaccg gcccagctcgtgggtgatcggcgcggcaccgcccgtacaacgcgagcccgcatcgc caacggcatgggcgcgaccgttacgggttctagacatcaacatcgacaaacttcggc aactcgacgcgagttctgcggccggatccacactcgctactcatcggcctacgag ctcgaggggtgcccgtcaaacgtgccgacctggtgattggggccgtcctggtgccagg cgccaaggcaccacaaattagtctcgaattcacttgcgcgcatatgaaaccagggtg cggtagctggatagccatcgaccagggcggtgtttcgaaggctcacgaccg accacctacgaccacccgacgttcgccgtgcacgacacgctgttttactgcgtggc gaacatgcccgcctcgggtgccgaagacgtcgacctacgcgtgacacacgcgacga tgccgtatgtgctcgagcttgccgacctgggtggcgggcggtgcccgtcgaat ccggcactagccaaaggctcttctgacgcacgaagggcggttactgtccgaacgggt ggccaccgacctgggggtgccgttcaccgagcccgccagcgtgctggcc

Table 2. (continued) DNA sequences of selected low oxygen induced antigens		
Rv no.	Seq ID no.	Sequence
Rv3126c	87	atgggtcatccgggtttgatcaaataggggtcattgggtcctctcaatgaaatcccttgc gtcactgtcgttttcagcgggtgtctgcgcgagaattctagtttgggtcgcggcgctgg accggctcgatgctgcgggtcgatgagctgagcgctttgtcgtttgatgcgttgacc actccggagcggggtcgcgcccggtcgcgacccgggaccatcatccttgggtcccgctc ccgctcgcagttgtgcgccagaaatggcgacgggtgcagtgcaccaatgccagtggc cgaaggcgggtttgggtgtcattgacaatcca
	88	gtgctcaagaacgcagtccttggctggcatgccgggcgccgtcgggtgcacaacagcca gccctggcggttgggtggccgaaagcggtcccgagcacactactgtgcacctgttcg tcaaccgccaccgaacgggtgccggccaccgaccattccggccggcaagcgatcatc agttgcggtgcggtactcgatcaccttcgcatcgccatgacggccgcgcactggca ggcgaatatcactcgttttcccagccgaaccaacctgaccagttggccaccgctcg aattcagtcgccatcgatcacgtcacggcgggacagcgaaaccgcgcccaggcgatt ctgcagcgcggaaccgatcggttccgtttgacagcccgatgtactggcacctgtt tgagcccgcgctgcgcgacgcggtcgacaaaagacgttgcgatgcttgatgtggtat ccgacgaccagcgaacacgactgggtggtagcgtcacaaactcagcgaagtccgtcgg cgggacgatccgtactatcacgccgaactcgaatgggtggacttcaccgttcgtgct ggcccatgggtgtgccgccggatagcgtggcatcagacgccgaacgcttgccgggttg acctgggcccgtgacttcccgggtccggagctaccagaatcgccgtgcccagctagct gatgaccgatcgaaagtccttgtgctgtcgacccctagcgacacgcgagccgacgc actgaggtgtggcgaagtgtgtcgaccatcctactcgagtgcaccatggccggca tggtacctgcacgttgacctatctgatcgaatccagtgcagctcgtgacatcgtg cggggcctgacgaggcagcgaggcgagccgcaagccttgatccgggtagggatagc cccgcggttggcagcagttcccgcggccacaccacggcgccgctggacagcgtct tgagattcgccagacgcccagaaagggcgtaatgcctcagatagaaatgcccg gaaacgggttgggttcagcccgct
Rv3128c	89	gtgtggtccgcctcgggtgggcagtcggggaagtatcttgcgcctcgatggtgct gcagcttgatgggttggaaacgtcacgggtgtgttggagtttggcgctgacgcctatg gcccggaggtgcgtgaggagctgttggcgatgagtgccggccagcatcgatcgttat ctgaagaccgcgaaggccaaagaccagatatcggggtgtgtcgacgacgaaacctc accactgctgcgtaattcgatcaaggttcgcagggccggcgatgaggtcgaggcgg agccgggttcttcgagggcgacaccgtcgccattgcgggtccgacgctcaaaggc gagttcgccacaccctgaacttgaccgacgtgcacatcggatgggtgttcaccog caccgtccgcaacaacgcccgtaccacatcctcgccgggtcaaagcttctgtca ccgagatcccgcatgggataacgggtttagatttcgacaacggcaccgtgtttctc aacaagccggtcatcagctgggcccggcgacaacgggtatctacttcaccogcttctg cccgtacaagaaaaaccactaggccaccatcgagtccaagaacaaccacctggtcc gcaagtacgcttctactaccgctatgacaccgcccagggaacgcgcgctgtcaac cggatgtggaagctggtcaacgaccgctcaactacctcaccocgaccatcaaacc gatcgggtatgccagcagcgcgacggccgcgcgcgacgctctacgatgcccac agacgccgctggaccggccactggccgcaaggggtgctctccgcccagcaggcc gacctgatcacctaccgagacagcctcaaccccgcccagatcgcccgcaaaatcgc cgacctgcagaaccgactcctcatcttggccaaggagaaaaccgagcagctctacc tcgtaacatccccaccgcccctaccgacatccacaaaggcatcctgatcaagg- cgggc
	90	gtggtgcaaggccgcacccgtgctgtttcgtaccgcggaggggcgccaaattatttc agccgtcgcgaagtgcgcggtggctttcgaggcgagaccacaacggttgccgagg gctggagcgtgatcgtcaaggttcgcgccaggtgctgacgacccgacgcgggggtc cgcaagccgaacgcgcccagttactaccgtggaccgcgacgctgaaacgtcactg tgtgcgggtgatcccggtgggagatcacggccgcccacttcaggttcggtccggaac cggaccgcagccagaccttgcctgcgaggcctcgtcacacaaccagcga
Rv3129		

Table 2. (continued) DNA sequences of selected low oxygen induced antigens		
Rv no.	Seq ID no.	Sequence
Rv3130c	91	atgaatcacctaacgacacttgacgccgggtttctcaaggcagaagacgtggatcg gcacgtgagtcctggcaatcggcgctctggcggtcatcgaggggcccggctcccgatc aggaagccttcttatcgtcgctcgctcaacgcctacgtccctgtaccgggttcggg cagcggttacgcctgcgccggttcgacctcggtgcacccaaatgggtggacgatcc cgacttcgatcttggcgctcatgtgtggcgcatcgccctgcccgggctggcaacg aagaccagttattcgagctgatcgccgatctgatggcgctcggttgaccgggggt cgaccgctgtgggaggtctgggtcatcgaaggcctggcgagacagcaagtggcgat cctgaccaaactgcaccactgcatggccgacggaatcgcgcgactcacctgctag ctgggctctccgatgaaagtatgagcgacagcttcgcgagcaacatccacacgacc atgcagtcgcaatccgcactctgtgcggcggggtggattccgtgtcaatccaagcga ggctgtgaccgcgtcgaccgcctgatggcaggcatcgttcgcgcggccaagggtg ccagtgaatcgcgccggcggtgctaagtcccgccgctcgctcggttgaaacgggccc atcagtgatttgctcgctacagcgagcaaaaggtccctctcgccgacgtcgaaca ggtgtgcccgaatttcgacgtcaccatcaatgatgttgcgcttgccgcgattacgg aaagctaccgcaacgtcctcatccagcggggtgagcgccctaggtttgattcgctg cgtacgctagtgcgggtctcgacgcgttccaacagcgctttgagcaagaccgataa ccgtgtttcgtaaatgctgcccacactgccggtggatcaagagaaccgctgcagc ggctgcggatcgctgcactcgcggtgactcgggccaaggcgggggacagagacaa ttcggaataactttgatggcgattgccaacgccttcggttcccatgaccgcatg ggcggtcgggctgttgatgcggctgcccgcagcggtgtgtgtcaccgtggcgacaa atgtgcccgggtccacgacggccgctgcagattatgggcagacgggtgcttgacct taccgggtttcgccgatcgcgatgcaactgcgcaccagtgctcgcgatgctcagcta cgccgacgacctgtacttcgggatcctggccgactacgacgtggttagcagatgccg gccagctggcgcgagggaattgaagacgccgtcgacggctggtggcgatcagtaag cggcgcaagggtgactcgacggcgcgagcgctatcgctggttggtg
	92	atgaacacccatttcccggacgcgaaaccgtgcgaacggttctcacctggcgt ccgggccccctccatccacaacacgcagccgtggcggtggcggttatgcccgacga gtctggagctgttctctagaccgatatgcagctgcgtagcaccgatccggacggg cgtgagttgatcctcagctgtggtgtggcattgcaccactgcgtcgctcgcttggc gtcgctgggctggcaggccaaggtaaacggtttccccgatccaaggacccgctgcc atctggccaccatcggggtacaaccgcttgttcccgatcaggccgatgtcgccctt gcggcgccataaccgcggcgacgcaccgatcggcgcgcctacagttgctggccggt gccaggaggtgacatcgcttgatggccgcaagagcagccgtggcggggtcatgc tgcggcaggtcagtgccctagaccgaatgaaagccattgtggcgaggtgtcttg gaccacgtgaccgacgaggaatatctgcgcgagctcaccatttggagtgggcgcta cggttcagtgggcggggttcccgcgcgcaacgagccgcatcagaccccagtgccc cgatccccggctgcctgttcgcggggcccggtctgtctcagccgtccgacgtctta ccgctgacgacggcgccgcgatcctggcactaggcaccgagacagacgacccggtt ggcccggctgcgcgcggcgaggccgcccagcatcgtcttgttgaccgcgacggcaa tggggctggcggtgctgcccgatcaccgaaccgctggagatcgccaagacccgcgac gcggctcggtgcccaggtgttcggcgccggcgctacccccagatgctgctgcgagt ggggtgggcacccgatcaatgccgacccgttgccaccgacgccacggcgcgaaactgt cccaggtcggttgagtgccgggaagagctactgcgacaacggtgc
Rv3131		

Table 2. (continued) DNA sequences of selected low oxygen induced antigens		
Rv no.	Seq ID no.	Sequence
Rv3132c	93	atgacaacagggggcctcgatcgacgaaaacgacggcgccgcaatgcgctccactgcg tcacacgctctcccaactacgcctgcacgagctgctggtcgaggtgcaggaccggg tcgagcagatcgatcgagggccgggaccgcctcgatggctggtggaggccatgctc gtggtcacagcgggctggacctggaggcaaccctacgcgctatcgatgcattcagc gaccagccttgcgacgctgctgagggcctatggcgctatggagggtgcacgaccggcagcatc gggatttgactttgtctatgaaggcatcgacgaggagaccggttcggcggtatcggc cacctaccgaaaggcctaggcgctcatcgggctgctcatcgaagatcccaaaccggt acggctggacgatgtttctgcgaccccgccctcgattgggtttcccgcgatcatc cgccgatgctaccttccctcggggtaccggttcgggtgctcgatgaatcggttcggc actctgtacctgactgacaagaccaacgggcaaccggttcagcgacgacgacgaggt tctggtccaggcgctggcgcccgccgggtatcgagtcgcgaatgcccggtctc accagcaggctaaggcgctcagtcggtggatcgaggccaccggtgacatcgccacc gagggtgttgcggcaccgaaccccgcgacgggtgttcgggttgcgcggcgaggc gctcaagctgacggcggtgacgctgccctggtagccggttcggctcgacgaggaca tgccctgccgctgacgtgggggagctgctggtgattgaaacagtcggcagcgctgtg gcttccattgttgggcgaacgattccgggtggcgggcgcggtgctgcgggagggtctt cgtcaacggcattcccgacgggtcgaccgggtcgatttggaaggcctggacgaac tggccgacgcagggtccggcgctgctgttgccgctgcgggccagaggtaccgtagcg ggtgtcggttgttgcgtgagtcaggcggtccaggggcttccaccgacgaacaact cgagatgatggccggttcgcccagaccagcccgctggcttggaattggccactt cgcaacgtcggtatgcgcgaactcgacgtactgaccgaccgggtatcgatcgccgt gacctccatgacctgtcatccagcggtcttcgcgattggcctggcttgcagggt tgctgtcccgcacgaacgtaatcctgaagtgcagcaacgactctcgacgtggtag acgatctgcaagacgttatcacaggaaatccggaccaccatttatgacctgcacgga gcatcgagggtatcactcggtccggcagcgaatcgatgcggccgtagcccaatt tgccgactcggggttgcgcaccagcggttcaattcggtgggtccattgtcggtggcg acagcgcgctcgccgatcaggccgaggcggtggttcgggaagcggtcagcaacgcg gttcgccatgcgaaggccagcacgttgaccgtccgggtcaaagtcgacgacgactt gtgcatcgagggtgaccgacaacggcccgggctgcccagcaggttcaccggaagcg gcttaacgaacctgcccagcgggcagagcaggccggcggaattcacccctcgcg agcgtaccgggcgcgagcgggaacagtgctgcgatggtcagcacccgttgcgcag
	94	gtggtaaaggctcttcttggatgaccacgaggtgggtgcgctcggtggtctggttga ctgcttggggccgatcccagcttgacgtcgtaggtgaggcggttcggctgcgcg aggcgatggccagggttccctgcccgcgcccagatgtcgcggtgctggatgtccgg ttgcccgatggcaacggcattgaactgtccgcgatctgttgcctccgatgccga tctgcgctgtctgactcctcacgtcctacacctctgacgaggccatgctagatgcga ttctcgccggtgccagcggtatgtcgctcaaagacatcaagggaatggagttggcg cgcgccgtcaaagatgtggcgctggacggtcgctgctggacaatcgggcccgggc cgcgctgatggccaagctgcgcggtgccgcccagaaagcaggaccgcctatcaggcc ttaccgaccaggagcgacgctactgggctgcttagcgagggcctgaccaacaag cagatcgccgaccgaatgttcctagccgaaaagacggtgaagaactacgtgtcgcg gttgctggccaagctgggcatggaacgtcgacgcaagcccggtattcgcgacgg agttgaagcgctcgcgccaccgggtgatggacca

Table 2. (continued) DNA sequences of selected low oxygen induced antigens		
Rv no.	Seq ID no.	Sequence
Rv3134c	95	atgagcgcacccctcgccagctcgggcagtggtcgttgggtatcgacgggtcaagggc ggcaacgcacgctcggttggggcggtcgatgaggcggtgaaccgagacattccgc tgcgactgggtgtacgtcatcgatccgtcccaactgtccgcgcggcgaggcggt gggcaatcagcggcccgagcggcgctgcacgacgcctctcggaaggctcaggccac cgggcaaccggtcaagatcgaaacggaggttctgtgcggcaggccgctcaccagc tgatgcaggagtcagggtccgcggcgatgctgtgcgtcggttcgggtggggcttgat catgtccgcggtcgccgggttcgggtcgcggcgaccctgggtgggtcggccttatg ccccgtggcggtgattcaccgcgtcgccggccgagccagcgacaacctcccaggta gcgcggttgtcgcggaggtggacaatgggtgtggtgctgcggcagcattcgaggag gccagggtgcgcggaggttccgctgcgggcggtgggtgtccacgctgctgaaacac cgatgacgtcgaaacaggcgagccggttggcgcatgtacacctgagccgtcggtcg ccactggaccgggtctaccccgaggtgcgggtggatcgggccatcgccggcggc agtgcgtgccgtcatctggccgcaacgcaaagccgggtcagctgttcgtcgcgga ctcacactccgcgcacgaattgtgcggtgcataccagcccgatgcgcgtaactta cggtacgcagtgccaacttg
	96	atgacagaatacgaaggccctaagacaaaattccacgcgttaatgcaggaacagat tcataacgaattcacagcggcacaacaatatgtcgcgatcgcggtttatttcgaca gcgaagacctgccgcagttggcgaagcatttttacagccaagcggtcgaggaacga aaccatgcaatgatgtcgtgcaacacctgctcgaccgcgaccttcgtgtcgaaat tcccggcgtagacacgggtgcgaaccagttcgacagaccccgagggcactggcgc tgccgctcgatcaggaacgcacagtcaccgaccaggtcggtcggtgacagcggtg gcccgcgacgagggcgatttccctcggcgagcagttcatgcagtggttcttgaggga acagatcgaagaggtggccttgatggcaaccttggtgcgggttgccgatcgggccg gggccaacctgttcgagctagagaacttcgtcgacgctgaagtggatgtggcgccg gccgcatcaggcgccccgcacgctgcgggggcccgcctc
Rv3841	97	atgacatgggcccgcagaggtgctcgcgcgacatccctttgtggttgcacccgtgg tgctcgccggtcgcgcggagcatacccttgcgcgctacgacctggcgctcaaag agggcgccgcagcggtggaatgtgatgtgcggttgaccgggagcgggcatctggtc tgtgtgcatgaccgcgcctggaccgaacctcgacgggagcgggcttggtcagcac gatgacgctggcccagctacgcgagctggagtagcgcgcgtggcagcagcagctggc gccccgacggttcgcacggcgacaccagctctgctgacctggacgcgcttggttcg ctggttttgactggcaccggccgggtgaagatcttcgtcgagaccaagcatcccgt ccgatacggctcgttggtggaacaaagctgctggcgctgctacaccggttcggta ttgccgcacccgcctccgcagatcgatcccggtgggtgagtgctggttttcggcc gccgcggtctggcggtacccggcggtgcaccgctgctgcccagcggtgttgctcgg caagaccccccgataacctgaccagcagtgccggccacggcggtcggggcaaccgcg tgggacctcactgctgcgttaaagggaatatccgcaactcgttgaccgctcgga gctcagggccggcggtgtactgctggaacgctcgatgagtagcaggacatcgactt ttgccgggaggtcggggtggcctggattggtactcaccaccccgccgcaccaagg cctggctggaagacgggcccggcgaacgggaccactcgc
	98	gtgtccgacggcgaacaagccaaatcacgtcgacgcggggggcgccgcggcg gcgcgctgcggctacagccgagaatcacatggacgccccacggccggcgacgcca ccccgaccccgcaacggcggaagcggtcccggtcccgctcacctcgtcgcggtcg actcggtatgcgcaccgtgcacgaacatcggtggaggggttggtcattgacgggtat cgacggtccacgagacgcgcaggtcgcggtctgatcgccgcgctcgaccggcgcg gccggctgctgtggtcgctacccaaggggcacatcgagttggggcgagaccgag cagaccgcatccgcgaggtcgccgaggagaccggcatccgcggcagtggtgctcgc cgcgctggggcgcatcgactactggttcgtcaccgacggccggcggtgcacaaga ccgtccaccattatttgatgcgggtttttaggcggagagctgcccgaagacctc gaggtagccgaggttagcctgggtgcccgatccgggaactgcgctctcgactggccta cgccgacgaacgtcgactagccgaggtggccgacgaactgatcgacaagctgcaga gcgacggccccgcgcgcttcgcgcgctaccacccagctcgccctcgtcgacggccg caaacgcattcacgcgctcgtcatgccgatgactcagcaccgggtcagcacaacgg tcccgggcccggggccg
Rv3842c	99	atgacatgggcccgcagaggtgctcgcgcgacatccctttgtggttgcacccgtgg tgctcgccggtcgcgcggagcatacccttgcgcgctacgacctggcgctcaaag agggcgccgcagcggtggaatgtgatgtgcggttgaccgggagcgggcatctggtc tgtgtgcatgaccgcgcctggaccgaacctcgacgggagcgggcttggtcagcac gatgacgctggcccagctacgcgagctggagtagcgcgcgtggcagcagcagctggc gccccgacggttcgcacggcgacaccagctctgctgacctggacgcgcttggttcg ctggttttgactggcaccggccgggtgaagatcttcgtcgagaccaagcatcccgt ccgatacggctcgttggtggaacaaagctgctggcgctgctacaccggttcggta ttgccgcacccgcctccgcagatcgatcccggtgggtgagtgctggttttcggcc gccgcggtctggcggtacccggcggtgcaccgctgctgcccagcggtgttgctcgg caagaccccccgataacctgaccagcagtgccggccacggcggtcggggcaaccgcg tgggacctcactgctgcgttaaagggaatatccgcaactcgttgaccgctcgga gctcagggccggcggtgtactgctggaacgctcgatgagtagcaggacatcgactt ttgccgggaggtcggggtggcctggattggtactcaccaccccgccgcaccaagg cctggctggaagacgggcccggcgaacgggaccactcgc
	100	gtgtccgacggcgaacaagccaaatcacgtcgacgcggggggcgccgcggcg gcgcgctgcggctacagccgagaatcacatggacgccccacggccggcgacgcca ccccgaccccgcaacggcggaagcggtcccggtcccgctcacctcgtcgcggtcg actcggtatgcgcaccgtgcacgaacatcggtggaggggttggtcattgacgggtat cgacggtccacgagacgcgcaggtcgcggtctgatcgccgcgctcgaccggcgcg gccggctgctgtggtcgctacccaaggggcacatcgagttggggcgagaccgag cagaccgcatccgcgaggtcgccgaggagaccggcatccgcggcagtggtgctcgc cgcgctggggcgcatcgactactggttcgtcaccgacggccggcggtgcacaaga ccgtccaccattatttgatgcgggtttttaggcggagagctgcccgaagacctc gaggtagccgaggttagcctgggtgcccgatccgggaactgcgctctcgactggccta cgccgacgaacgtcgactagccgaggtggccgacgaactgatcgacaagctgcaga gcgacggccccgcgcgcttcgcgcgctaccacccagctcgccctcgtcgacggccg caaacgcattcacgcgctcgtcatgccgatgactcagcaccgggtcagcacaacgg tcccgggcccggggccg
Rv3908	101	atgacatgggcccgcagaggtgctcgcgcgacatccctttgtggttgcacccgtgg tgctcgccggtcgcgcggagcatacccttgcgcgctacgacctggcgctcaaag agggcgccgcagcggtggaatgtgatgtgcggttgaccgggagcgggcatctggtc tgtgtgcatgaccgcgcctggaccgaacctcgacgggagcgggcttggtcagcac gatgacgctggcccagctacgcgagctggagtagcgcgcgtggcagcagcagctggc gccccgacggttcgcacggcgacaccagctctgctgacctggacgcgcttggttcg ctggttttgactggcaccggccgggtgaagatcttcgtcgagaccaagcatcccgt ccgatacggctcgttggtggaacaaagctgctggcgctgctacaccggttcggta ttgccgcacccgcctccgcagatcgatcccggtgggtgagtgctggttttcggcc gccgcggtctggcggtacccggcggtgcaccgctgctgcccagcggtgttgctcgg caagaccccccgataacctgaccagcagtgccggccacggcggtcggggcaaccgcg tgggacctcactgctgcgttaaagggaatatccgcaactcgttgaccgctcgga gctcagggccggcggtgtactgctggaacgctcgatgagtagcaggacatcgactt ttgccgggaggtcggggtggcctggattggtactcaccaccccgccgcaccaagg cctggctggaagacgggcccggcgaacgggaccactcgc
	102	gtgtccgacggcgaacaagccaaatcacgtcgacgcggggggcgccgcggcg gcgcgctgcggctacagccgagaatcacatggacgccccacggccggcgacgcca ccccgaccccgcaacggcggaagcggtcccggtcccgctcacctcgtcgcggtcg actcggtatgcgcaccgtgcacgaacatcggtggaggggttggtcattgacgggtat cgacggtccacgagacgcgcaggtcgcggtctgatcgccgcgctcgaccggcgcg gccggctgctgtggtcgctacccaaggggcacatcgagttggggcgagaccgag cagaccgcatccgcgaggtcgccgaggagaccggcatccgcggcagtggtgctcgc cgcgctggggcgcatcgactactggttcgtcaccgacggccggcggtgcacaaga ccgtccaccattatttgatgcgggtttttaggcggagagctgcccgaagacctc gaggtagccgaggttagcctgggtgcccgatccgggaactgcgctctcgactggccta cgccgacgaacgtcgactagccgaggtggccgacgaactgatcgacaagctgcaga gcgacggccccgcgcgcttcgcgcgctaccacccagctcgccctcgtcgacggccg caaacgcattcacgcgctcgtcatgccgatgactcagcaccgggtcagcacaacgg tcccgggcccggggccg



Preferably the immunogenic portions are selected from the group consisting of the sequences presented in Table 1 and the nucleic acid sequences are selected from the sequences presented in Table 2.

5

In another embodiment, the vaccine is a multiphase vaccine, where the polypeptides or fragments hereof are fused to other antigens with efficacy as prophylactic vaccines, where the fusionpartner is selected from eg. the group consisting of ESAT-6, TB10.4, CFP10, RD1-ORF5, RD1-ORF2, Rv1036, MPB64, MPT64, Ag85A, Ag85B (MPT59), MPB59,

10 Ag85C, 19kDa lipoprotein, MPT32.

The invention further discloses a therapeutic vaccine against tuberculosis comprising one or more polypeptides or fragments hereof, which polypeptides are expressed during the latent stage of the mycobacterial infection, which stage is characterized by low-oxygen  
15 tension in the microenvironment of the mycobacteria, or nucleic acids encoding these polypeptides.

Preferably, the therapeutic and multiphase vaccine comprises an additional delivery system selected among, live recombinant vaccines, that is gene-modified organisms such as  
20 bacteria or viruses expressing mycobacterial genes, or immunogenic delivery systems such as, DNA vaccines, that is plasmids expressing genes or gene fragments for the proteins described above, or protein vaccines, that is the proteins themselves or synthetic peptides derived from the proteins themselves delivered in a delivery system such as an adjuvant.

25 The invention further discloses a therapeutic vaccine in which the amino acid sequence is lipidated so as to allow a self-adjuvanting effect of the polypeptide.

The invention also discloses a method for treating an animal, including a human being, with tuberculosis caused by virulent mycobacteria, e.g. by *Mycobacterium tuberculosis*,  
30 *Mycobacterium africanum* or *Mycobacterium bovis*, comprising administering to the animal the above-mentioned vaccine.

The invention also discloses a method for immunising an animal, including a human being, against tuberculosis caused by virulent mycobacteria, e.g. by *Mycobacterium tuberculosis*,  
35 *Mycobacterium africanum* or *Mycobacterium bovis*, comprising administering to the animal the above mentioned vaccine.

In a still further embodiment, the invention discloses an immunogenic composition comprising a polypeptide as defined above, preferably in the form of a vaccine or in the form of a diagnostic reagent. The diagnostic reagent can be in the form of a skin test reagent  
 5 (administered by the transcutaneous, subcutaneous or intradermal routes), a serological reagent or a reagent for stimulating a cell-mediated reaction.

In another embodiment, the invention discloses a nucleic acid fragment in isolated form which

- 10 (a) comprises a nucleic acid sequence which encodes a polypeptide as defined above, or comprises a nucleic acid sequence complementary thereto; or
- (b) has a length of at least 10 nucleotides and hybridizes readily under stringent hybridization conditions with a nucleotide sequence selected from the nucleotide sequences presented in Table 1 or a sequence complementary thereto, or with a nu-  
 15 cleotide sequence selected from a sequence in (a)

The nucleic acid fragment is preferably a DNA fragment. The fragment can be used as a pharmaceutical.

- 20 In another embodiment, the invention discloses a vaccine comprising a nucleic acid fragment according to the invention, optionally inserted in a vector, the vaccine effecting *in vivo* expression of antigen by an animal, including a human being, to whom the vaccine has been administered, the amount of expressed antigen being effective to confer substantially increased resistance to tuberculosis caused by virulent mycobacteria, e.g. by  
 25 *Mycobacterium tuberculosis*, *Mycobacterium africanum* or *Mycobacterium bovis*, in an animal, including a human being.

- In a further embodiment, the invention discloses the use of a nucleic acid fragment according to the invention for the preparation of a composition for the diagnosis of tubercu-  
 30 losis caused by virulent mycobacteria, e. g. by *Mycobacterium tuberculosis*, *Mycobacterium africanum* or *Mycobacterium bovis*, and the use of a nucleic acid fragment according to the invention for the preparation of a pharmaceutical composition for the vaccination against tuberculosis caused by virulent mycobacteria, e.g. by *Mycobacterium tuberculo-*  
*sis*, *Mycobacterium africanum* or *Mycobacterium bovis*.

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- In a still further embodiment, the invention discloses a vaccine for immunizing an animal, including a human being, against tuberculosis caused by virulent mycobacteria, e.g. by *Mycobacterium tuberculosis*, *Mycobacterium africanum* or *Mycobacterium bovis*, comprising as the effective component a non-pathogenic microorganism, wherein at least one
- 5 copy of a DNA fragment comprising a DNA sequence encoding a polypeptide as defined above has been incorporated into the microorganism (e.g. placed on a plasmid or in the genome) in a manner allowing the microorganism to express and optionally secrete the polypeptide.
- 10 In another embodiment, the invention discloses a replicable expression vector, which comprises a nucleic acid fragment according to the invention, and a transformed cell harbouring at least one such vector.

In another embodiment, the invention discloses a method for producing a polypeptide as

15 defined above, comprising

- (a) inserting a nucleic acid fragment according to the invention into a vector which is able to replicate in a host cell, introducing the resulting recombinant vector into the host cell, culturing the host cell in a culture medium under conditions sufficient to effect expression of the polypeptide, and recovering the polypeptide from the

20 host cell or culture medium;

- (b) isolating the polypeptide from a whole mycobacterium, e.g. *Mycobacterium tuberculosis*, *Mycobacterium africanum* or *Mycobacterium bovis*, from culture filtrate or from lysates or fractions thereof; or
- (c) synthesizing the polypeptide e.g. by solid or liquid phase peptide synthesis.

25

The invention also discloses a method of diagnosing tuberculosis caused by virulent mycobacteria, e.g. by *Mycobacterium tuberculosis*, *Mycobacterium africanum* or *Mycobacterium bovis*, in an animal, including a human being, comprising intradermally injecting, in the animal, a polypeptide as defined above or an immunogenic composition as de-

30 fined above, a positive skin response at the location of injection being indicative of the animal having tuberculosis, and a negative skin response at the location of injection being indicative of the animal not having tuberculosis.

In another embodiment, the invention discloses a method for immunising an animal, including a human being, against tuberculosis caused by virulent mycobacteria, e.g. by *My-*

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*cobacterium tuberculosis*, *Mycobacterium africanum* or *Mycobacterium bovis*, comprising administering to the animal the polypeptide as defined above, the immunogenic composition according to the invention, or the vaccine according to the invention.

- 5 Another embodiment of the invention discloses a monoclonal or polyclonal antibody, which is specifically reacting with a polypeptide as defined above in an immuno assay, or a specific binding fragment of said antibody. Preferably, said antibody is for use as a diagnostic reagent, e.g. for detection of mycobacterial antigens in sputum, urine or other body fluids of an infected animal, including a human being.

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In a further embodiment the invention discloses a pharmaceutical composition which comprises an immunologically responsive amount of at least one member selected from the group consisting of:

- (a) a polypeptide selected from the sequences presented in Table 1, or an immunogenic portion thereof;
- 15 (b) an amino acid sequence which has a sequence identity of at least 70% to any one of said polypeptides in (a) and is immunogenic;
- (c) a fusion polypeptide comprising at least one polypeptide or amino acid sequence according to (a) or (b) and at least one fusion partner;
- 20 (d) a nucleic acid sequence which encodes a polypeptide or amino acid sequence according to (a), (b) or (c);
- (e) a nucleic acid sequence which is complementary to a sequence according to (d);
- (f) a nucleic acid sequence which has a length of at least 10 nucleotides and which hybridizes under stringent conditions with a nucleic acid sequence according to
- 25 (d) or (e); and
- (g) a non-pathogenic micro-organism which has incorporated (e.g. placed on a plasmid or in the genome) therein a nucleic acid sequence according to (d), (e) or (f) in a manner to permit expression of a polypeptide encoded thereby.

- 30 In a still further embodiment the invention discloses a method for stimulating an immunogenic response in an animal which comprises administering to said animal an immunologically stimulating amount of at least one member selected from the group consisting of:

- (a) a polypeptide selected from from the sequences presented in Table 1, or an immunogenic portion thereof;
- 35

- (b) an amino acid sequence which has a sequence identity of at least 70% to any one of said polypeptides in (a) and is immunogenic;
  - (c) a fusion polypeptide comprising at least one polypeptide or amino acid sequence according to (a) or (b) and at least one fusion partner;
  - 5 (d) a nucleic acid sequence which encodes a polypeptide or amino acid sequence according to (a), (b) or (c);
  - (e) a nucleic acid sequence which is complementary to a sequence according to (d);
  - (f) a nucleic acid sequence which has a length of at least 10 nucleotides and which hybridizes under stringent conditions with a nucleic acid sequence according to
  - 10 (d) or (e); and
  - (g) a non-pathogenic micro-organism which has incorporated therein (e.g. placed on a plasmid or in the genome) a nucleic acid sequence according to (d), (e) or (f) in a manner to permit expression of a polypeptide encoded thereby.
- 15 The vaccine, immunogenic composition and pharmaceutical composition according to the invention can be used therapeutically in a subject infected with a virulent mycobacterium combined with a prophylactic composition in a subject to prevent further infection with a virulent mycobacterium.
- 20 The invention also discloses a method for diagnosing previous or ongoing infection with a virulent mycobacterium, said method comprising
- (a) contacting a sample, e.g. a blood sample, with a composition comprising an antibody according to the invention, a nucleic acid fragment according to the invention and/or a polypeptide as defined above, or
  - 25 (b) contacting a sample, e.g. a blood sample comprising mononuclear cells (e.g. T-lymphocytes), with a composition comprising one or more polypeptides as defined above in order to detect a positive reaction, e.g. proliferation of the cells or release of cytokines such as IFN- $\gamma$ .
- 30 Finally, the invention discloses a method of diagnosing *Mycobacterium tuberculosis* infection in a subject comprising:
- (a) contacting a polypeptide as defined above with a bodily fluid of the subject;
  - (b) detecting binding of a antibody to said polypeptide, said binding being an indication that said subject is infected by *Mycobacterium tuberculosis* or is susceptible
  - 35 to *Mycobacterium tuberculosis* infection.

## Definitions

### *Polypeptides*

The word "polypeptide" in the present invention should have its usual meaning. That is an amino acid chain of any length, including a full-length protein, oligopeptides, short peptides and fragments thereof, wherein the amino acid residues are linked by covalent peptide bonds.

The polypeptide may be chemically modified by being glycosylated, by being lipidated (e.g. by chemical lipidation with palmitoyloxy succinimide as described by Mowat et al. 1991 or with dodecanoyl chloride as described by Lustig et al. 1976), by comprising prosthetic groups, or by containing additional amino acids such as e.g. a his-tag or a signal peptide.

Each polypeptide may thus be characterised by specific amino acids and be encoded by specific nucleic acid sequences. It will be understood that such sequences include analogues and variants produced by recombinant or synthetic methods wherein such polypeptide sequences have been modified by substitution, insertion, addition or deletion of one or more amino acid residues in the recombinant polypeptide and still be immunogenic in any of the biological assays described herein. Substitutions are preferably "conservative". These are defined according to the following table. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other. The amino acids in the third column are indicated in one-letter code.

ALIPHATIC	Non-polar	GAP
		ILV
	Polar-uncharged	CSTM
		NQ
	Polar-charged	DE
		KR
AROMATIC		HFWY

- A preferred polypeptide within the present invention is an immunogenic antigen from *M. tuberculosis* produced when the organism is subjected to the stresses associated with latent infection. Such antigen can for example also be derived from the *M. tuberculosis* cell and/or *M. tuberculosis* culture filtrate. Thus, a polypeptide comprising an immunogenic
- 5 portion of one of the above antigens may consist entirely of the immunogenic portion, or may contain additional sequences. The additional sequences may be derived from the native *M. tuberculosis* antigen or be heterologous and such sequences may, but need not, be immunogenic.
- 10 Each polypeptide is encoded by a specific nucleic acid sequence. It will be understood that such sequences include analogues and variants hereof wherein such nucleic acid sequences have been modified by substitution, insertion, addition or deletion of one or more nucleic acid. Substitutions are preferably silent substitutions in the codon usage which will not lead to any change in the amino acid sequence, but may be introduced to
- 15 enhance the expression of the protein.

- In the present context the term "substantially pure polypeptide fragment" means a polypeptide preparation which contains at most 5% by weight of other polypeptide material with which it is natively associated (lower percentages of other polypeptide material are
- 20 preferred, e.g. at most 4%, at most 3%, at most 2%, at most 1%, and at most ½%). It is preferred that the substantially pure polypeptide is at least 96% pure, *i.e.* that the polypeptide constitutes at least 96% by weight of total polypeptide material present in the preparation, and higher percentages are preferred, such as at least 97%, at least 98%, at least 99%, at least 99,25%, at least 99,5%, and at least 99,75%. It is especially preferred
- 25 that the polypeptide fragment is in "essentially pure form", *i.e.* that the polypeptide fragment is essentially free of any other antigen with which it is natively associated, *i.e.* free of any other antigen from bacteria belonging to the tuberculosis complex or a virulent mycobacterium. This can be accomplished by preparing the polypeptide fragment by means of recombinant methods in a non-mycobacterial host cell as will be described in detail below,
- 30 or by synthesizing the polypeptide fragment by the well-known methods of solid or liquid phase peptide synthesis, e.g. by the method described by Merrifield or variations thereof.

By the term "virulent mycobacterium" is understood a bacterium capable of causing the tuberculosis disease in an animal or in a human being. Examples of virulent mycobacteria include but are not limited to *M. tuberculosis*, *M. africanum*, and *M. bovis*. Examples of relevant animals are cattle, possums, badgers and kangaroos.

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By "a TB patient" is understood an individual with culture or microscopically proven infection with virulent mycobacteria, and/or an individual clinically diagnosed with TB and who is responsive to anti-TB chemotherapy. Culture, microscopy and clinical diagnosis of TB are well known by any person skilled in the art.

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By the term "PPD-positive individual" is understood an individual with a positive Mantoux test or an individual where PPD induces a positive *in vitro* recall response determined by release of IFN- $\gamma$ .

- 15 By "a latently infected individual" is understood an individual, who has been exposed to *M. tuberculosis* and converted to become PPD-positive but has never received anti-TB treatment nor is BCG vaccinated. It is likely that individuals who have been vaccinated or treated for TB may still retain the mycobacteria within their bodies, although this is currently impossible to prove since such individuals would be expected to be positive if tested  
20 for PPD reactivity. Nonetheless, in its most accurate sense, "latently-infected" may be used to describe any individual who has *M. tuberculosis* residing in their tissues but who is not clinically ill.

- By the term "delayed type hypersensitivity reaction" (DTH) is understood a T-cell mediated inflammatory response elicited after the injection of a polypeptide into, or application  
25 to, the skin, said inflammatory response appearing 72-96 hours after the polypeptide injection or application.

- By the term "IFN- $\gamma$ " is understood interferon-gamma. The measurement of IFN- $\gamma$  is used  
30 as an indication of an immunological response.

- By the terms "nucleic acid fragment" and "nucleic acid sequence" are understood any nucleic acid molecule including DNA, RNA, LNA (locked nucleic acids), PNA, RNA, dsRNA and RNA-DNA-hybrids. Also included are nucleic acid molecules comprising non-naturally  
35 occurring nucleosides. The term includes nucleic acid molecules of any length e.g. from



10 to 10000 nucleotides, depending on the use. When the nucleic acid molecule is for use as a pharmaceutical, e.g. in DNA therapy, or for use in a method for producing a polypeptide according to the invention, a molecule encoding at least one epitope is preferably used, having a length from about 18 to about 1000 nucleotides, the molecule being optionally inserted into a vector. When the nucleic acid molecule is used as a probe, as a primer or in antisense therapy, a molecule having a length of 10-100 is preferably used. According to the invention, other molecule lengths can be used, for instance a molecule having at least 12, 15, 21, 24, 27, 30, 33, 36, 39, 42, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500 or 1000 nucleotides (or nucleotide derivatives), or a molecule having at most 10 10000, 5000, 4000, 3000, 2000, 1000, 700, 500, 400, 300, 200, 100, 50, 40, 30 or 20 nucleotides (or nucleotide derivatives).

The term "stringent" when used in conjunction with hybridization conditions is as defined in the art, i.e. the hybridization is performed at a temperature not more than 15-20°C under the melting point  $T_m$ , cf. Sambrook et al, 1989, pages 11.45-11.49. Preferably, the conditions are "highly stringent", i.e. 5-10°C under the melting point  $T_m$ .

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations thereof such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

#### *Sequence identity*

The term "sequence identity" indicates a quantitative measure of the degree of homology between two amino acid sequences of equal length or between two nucleotide sequences of equal length. The two sequences to be compared must be aligned to best possible fit allowing the insertion of gaps or alternatively, truncation at the ends of the protein sequences. The sequence identity can be calculated as  $\frac{(N_{ref} - N_{dif})100}{N_{ref}}$ , wherein  $N_{dif}$  is the total number of non-identical residues in the two sequences when aligned and wherein  $N_{ref}$  is the number of residues in one of the sequences. Hence, the DNA sequence AGTCAGTC will have a sequence identity of 75% with the sequence AATCAATC ( $N_{dif}=2$  and  $N_{ref}=8$ ). A gap is counted as non-identity of the specific residue(s), i.e. the DNA sequence AGTGTC will have a sequence identity of 75% with the DNA sequence AGTCAGTC ( $N_{dif}=2$  and  $N_{ref}=8$ ). Sequence identity can alternatively be calculated by the BLAST program e.g. the BLASTP program (Pearson, 1988, or [www.ncbi.nlm.nih.gov/cgi-bin/BLAST](http://www.ncbi.nlm.nih.gov/cgi-bin/BLAST)). In one as-

pect of the invention, alignment is performed with the sequence alignment method ClustalW with default parameters as described by Thompson J., *et al* 1994, available at <http://www2.ebi.ac.uk/clustalw/>.

- 5 A preferred minimum percentage of sequence identity is at least 80%, such as at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, and at least 99.5%.

*Immunogenic portion*

- 10 In a preferred embodiment of the invention, the polypeptide comprises an immunogenic portion of the polypeptide, such as an epitope for a B-cell or T-cell.

The immunogenic portion of a polypeptide is a part of the polypeptide, which elicits an immune response in an animal or a human being, and/or in a biological sample determined by any of the biological assays described herein. The immunogenic portion of a polypeptide may be a T-cell epitope or a B-cell epitope. Immunogenic portions can be re-  
15 lated to one or a few relatively small parts of the polypeptide, they can be scattered throughout the polypeptide sequence or be situated in specific parts of the polypeptide. For a few polypeptides epitopes have even been demonstrated to be scattered throughout the polypeptide covering the full sequence (Ravn et al 1999).

- In order to identify relevant T-cell epitopes which are recognised during an immune re-  
20 sponse, it is possible to use overlapping oligopeptides for the detection of MHC class II epitopes, preferably synthetic, having a length of e.g. 20 amino acid residues derived from the polypeptide. These peptides can be tested in biological assays (e.g. the IFN- $\gamma$  assay as described herein) and some of these will give a positive response (and thereby be immunogenic) as evidence for the presence of a T cell epitope in the peptide. For the detec-  
25 tion of MHC class I epitopes it is possible to predict peptides that will bind (Stryhn et al. 1996) and hereafter produce these peptides synthetic and test them in relevant biological assays e.g. the IFN- $\gamma$  assay as described herein. The peptides preferably having a length of e.g. 8 to 11 amino acid residues derived from the polypeptide. B-cell epitopes can be determined by analysing the B cell recognition to overlapping peptides covering the poly-  
30 peptide of interest as e.g. described in Harboe et al 1998.

Although the minimum length of a T-cell epitope has been shown to be at least 6 amino acids, it is normal that such epitopes are constituted of longer stretches of amino acids. Hence, it is preferred that the polypeptide fragment of the invention has a length of at  
35 least 7 amino acid residues, such as at least 8, at least 9, at least 10, at least 12, at least

14, at least 16, at least 18, at least 20, at least 22, at least 24, and at least 30 amino acid residues. Hence, in important embodiments of the inventive method, it is preferred that the polypeptide fragment has a length of at most 50 amino acid residues, such as at most 40, 35, 30, 25, and 20 amino acid residues. It is expected that the peptides having a  
 5 length of between 10 and 20 amino acid residues will prove to be most efficient as MHC class II epitopes and therefore especially preferred lengths of the polypeptide fragment used in the inventive method are 18, such as 15, 14, 13, 12 and even 11 amino acid residues. It is expected that the peptides having a length of between 7 and 12 amino acid residues will prove to be most efficient as MHC class I epitopes and therefore especially  
 10 preferred lengths of the polypeptide fragment used in the inventive method are 11, such as 10, 9, 8 and even 7 amino acid residues.

Immunogenic portions of polypeptides may be recognised by a broad part (high frequency) or by a minor part (low frequency) of the genetically heterogeneous human population. In addition some immunogenic portions induce high immunological responses  
 15 (dominant), whereas others induce lower, but still significant, responses (subdominant). High frequency > low frequency can be related to the immunogenic portion binding to widely distributed MHC molecules (HLA type) or even by multiple MHC molecules (Sini-gaglia, 1988, Kilgus, 1991).

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In the context of providing candidate molecules for a new vaccine against tuberculosis, the subdominant epitopes are however as relevant as are the dominant epitopes since it has been shown (Olsen, 2000) that such epitopes can induce protection regardless of the fact that they are not as strongly or broadly recognised.

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#### *Variants*

A common feature of the polypeptides of the invention is their capability to induce an immunological response as illustrated in the examples. It is understood that a variant of a polypeptide of the invention produced by substitution, insertion, addition or deletion may  
 30 also be immunogenic as determined by any of the assays described herein.

#### *Immune individual*

An immune individual is defined as a person or an animal, which has cleared or controlled an infection with virulent mycobacteria or has received a vaccination with *M.bovis* BCG.

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### *Immune response*

The immune response may be monitored by one of the following methods:

- 5                   • An in vitro cellular response is determined by induction of the release of a relevant cytokine such as IFN- $\gamma$  from, or the induction of proliferation in lymphocytes with-

10                   drawn from an animal or human being currently or previously infected with virulent mycobacteria or immunized with the relevant polypeptide. The induction being per-

15                   formed by the addition of the polypeptide or the immunogenic portion of the poly-

20                   peptide to a suspension comprising from  $2 \times 10^5$  cells to  $4 \times 10^5$  cells per well. The

25                   cells being isolated from either the blood, the spleen, the liver or the lung and the

30                   addition of the polypeptide or the immunogenic portion resulting in a concentration

35                   of not more than 20  $\mu\text{g}$  per ml suspension and the stimulation being performed

                    from two to five days. For monitoring cell proliferation the cells are pulsed with ra-

                    dioactive labeled Thymidine and after 16-22 hours of incubation detecting the pro-

                    liferation by liquid scintillation counting. A positive response is defined as being a

                    response more than background plus two standard deviations. The release of IFN-

$\gamma$  can be determined by the ELISA method, which is well known to a person skilled

                    in the art. A positive response being a response more than background plus two

                    standard deviations. Other cytokines than IFN- $\gamma$  could be relevant when monitoring

                    the immunological response to the polypeptide, such as IL-12, TNF- $\alpha$ , IL-4, IL-5,

                    IL-10, IL-6, TGF- $\beta$ . Another and more sensitive method for detecting the immune

                    response is the ELISpot method, in which the frequency of IFN- $\gamma$  producing cells is

                    determined. In an ELISpot plate (MAHA, Millipore) precoated with anti-murine IFN-

$\gamma$  antibodies (PharMingen) graded numbers of cells isolated from either blood,

                    spleen, or lung (typically between 1 to  $4 \times 10^5$  cells /well) are incubated for 24-32

                    hrs in the presence of the polypeptide or the immunogenic portion resulting in a

                    concentration of not more than 20  $\mu\text{g}$  per ml. The plates are subsequently incu-

                    bated with biotinylated anti-IFN- $\gamma$  antibodies followed by a streptavidin-alkaline

                    phosphatase incubation. The IFN- $\gamma$  producing cells are identified by adding

                    BCIP/NBT (Sigma), the relevant substrate giving rise to spots. These spots can be

                    enumerated using a dissection microscope. It is also a possibility to determine the

                    presence of mRNA coding for the relevant cytokine by the use of the PCR tech-

                    nique. Usually one or more cytokines will be measured utilizing for example PCR,

                    ELISPOT or ELISA. It will be appreciated by a person skilled in the art that a sig-

                    nificant increase or decrease in the amount of any of these cytokines induced by a

specific polypeptide can be used in evaluation of the immunological activity of the polypeptide.

- 5      • An *in vitro* cellular response may also be determined by the use of T cell lines derived from an immune individual or an *M. tuberculosis*-infected person where the T cell lines have been driven with either live mycobacteria, extracts from the bacterial cell or culture filtrate for 10 to 20 days with the addition of IL-2. The induction being performed by addition of not more than 20 µg polypeptide per ml suspension to the T cell lines containing from  $1 \times 10^5$  cells to  $3 \times 10^5$  cells per well and incubation being performed from two to six days. The induction of IFN-γ or release of another relevant cytokine is detected by ELISA. The stimulation of T cells can also be monitored by detecting cell proliferation using radioactively labeled Thymidine as described above. For both assays a positive response being a response more than background plus two standard deviations.
- 15
- 20      • An *in vivo* cellular response may be determined as a positive DTH response after intradermal injection or local application patch of at most 100 µg of the polypeptide or the immunogenic portion to an individual who is clinically or subclinically infected with a virulent mycobacterium, a positive response having a diameter of at least 5 mm 72-96 hours after the injection or application.
- 25      • An *in vitro* humoral response is determined by a specific antibody response in an immune or infected individual. The presence of antibodies may be determined by an ELISA technique or a Western blot where the polypeptide or the immunogenic portion is absorbed to either a nitrocellulose membrane or a polystyrene surface. The serum is preferably diluted in PBS from 1:10 to 1:100 and added to the absorbed polypeptide and the incubation being performed from 1 to 12 hours. By the use of labeled secondary antibodies the presence of specific antibodies can be determined by measuring the OD e.g. by ELISA where a positive response is a response of more than background plus two standard deviations or alternatively a visual response in a Western blot.
- 30

- Another relevant parameter is measurement of the protection in animal models induced after vaccination with the polypeptide in an adjuvant or after DNA vaccination. Suitable animal models include primates, guinea pigs or mice, which are challenged with an infection of a virulent *Mycobacterium*. Readout for induced protection could be decrease of the bacterial load in target organs compared to non-vaccinated animals, prolonged survival times compared to non-vaccinated animals and diminished weight loss compared to non-vaccinated animals.

#### *Preparation methods*

- 10 In general, *M. tuberculosis* antigens, and DNA sequences encoding such antigens, may be prepared using any one of a variety of procedures.
- They may be purified as native proteins from the *M. tuberculosis* cell or culture filtrate by procedures such as those described above. Immunogenic antigens may also be produced recombinantly using a DNA sequence encoding the antigen, which has been inserted into
- 15 an expression vector and expressed in an appropriate host. Examples of host cells are *E. coli*. The polypeptides or immunogenic portion hereof can also be produced synthetically having fewer than about 100 amino acids, and generally fewer than 50 amino acids and may be generated using techniques well known to those ordinarily skilled in the art, such as commercially available solid-phase techniques where amino acids are sequentially
- 20 added to a growing amino acid chain.

- In the construction and preparation of plasmid DNA encoding the polypeptide as defined for DNA vaccination a host strain such as *E. coli* can be used. Plasmid DNA can then be prepared from cultures of the host strain carrying the plasmid of interest, and purified using e.g. the Qiagen Giga -Plasmid column kit (Qiagen, Santa Clarita, CA, USA) including
- 25 an endotoxin removal step. It is preferred that plasmid DNA used for DNA vaccination is endotoxin free.

#### *Fusion proteins*

- The immunogenic polypeptides may also be produced as fusion proteins, by which methods superior characteristics of the polypeptide of the invention can be achieved. For instance, fusion partners that facilitate export of the polypeptide when produced recombinantly, fusion partners that facilitate purification of the polypeptide, and fusion partners which enhance the immunogenicity of the polypeptide fragment of the invention are all interesting possibilities. Therefore, the invention also pertains to a fusion polypeptide
- 30

- comprising at least one polypeptide or immunogenic portion defined above and at least one fusion partner. The fusion partner can, in order to enhance immunogenicity, be another polypeptide derived from *M. tuberculosis*, such as of a polypeptide fragment derived from a bacterium belonging to the tuberculosis complex, such as ESAT-6, TB10.4,
- 5 CFP10, RD1-ORF5, RD1-ORF2, Rv1036, MPB64, MPT64, Ag85A, Ag85B (MPT59), MPB59, Ag85C, 19kDa lipoprotein, MPT32 and alpha-crystallin, or at least one T-cell epitope of any of the above mentioned antigens (Skj t et al 2000; Danish Patent application PA 2000 00666; Danish Patent application PA 1999 01020; US patent application 09/0505,739; Rosenkrands *et al* 1998; Nagai et al 1991). The invention also pertains to a
- 10 fusion polypeptide comprising mutual fusions of two or more of the polypeptides (or immunogenic portions thereof) of the invention.
- Other fusion partners, which could enhance the immunogenicity of the product, are lymphokines such as IFN- , IL-2 and IL-12. In order to facilitate expression and/or purification, the fusion partner can e.g. be a bacterial fimbrial protein, e.g. the pilus components pilin
- 15 and papA; protein A; the ZZ-peptide (ZZ-fusions are marketed by Pharmacia in Sweden); the maltose binding protein; glutathione S-transferase;  -galactosidase; or poly-histidine. Fusion proteins can be produced recombinantly in a host cell, which could be *E. coli*, and it is a possibility to induce a linker region between the different fusion partners.
- 20 Other interesting fusion partners are polypeptides, which are lipidated so that the immunogenic polypeptide is presented in a suitable manner to the immune system. This effect is e.g. known from vaccines based on the *Borrelia burgdorferi* OspA polypeptide as described in e.g. WO 96/40718 A or vaccines based on the *Pseudomonas aeruginosa* OprI lipoprotein (Cote-Sierra J 1998). Another possibility is N-terminal fusion of a known signal
- 25 sequence and an N-terminal cysteine to the immunogenic polypeptide. Such a fusion results in lipidation of the immunogenic polypeptide at the N-terminal cysteine, when produced in a suitable production host.

### Uses

#### Protein Vaccine

- 30 Another part of the invention pertains to a vaccine composition comprising a polypeptide (or at least one immunogenic portion thereof) or fusion polypeptide according to the invention. In order to ensure optimum performance of such a vaccine composition it is preferred that it comprises an immunologically and pharmaceutically acceptable carrier, vehicle or adjuvant.

An effective vaccine, wherein a polypeptide of the invention is recognized by the animal, will in an animal model be able to decrease bacterial load in target organs, prolong survival times and/or diminish weight loss after challenge with a virulent *Mycobacterium*,  
 5 compared to non-vaccinated animals

Suitable carriers are selected from the group consisting of a polymer to which the polypeptide(s) is/are bound by hydrophobic non-covalent interaction, such as a plastic, e.g. polystyrene, or a polymer to which the polypeptide(s) is/are covalently bound, such as a  
 10 polysaccharide, or a polypeptide, e.g. bovine serum albumin, ovalbumin or keyhole limpet haemocyanin. Suitable vehicles are selected from the group consisting of a diluent and a suspending agent. The adjuvant is preferably selected from the group consisting of dimethyldioctadecylammonium bromide (DDA), Quil A, poly I:C, aluminium hydroxide, Freund's incomplete adjuvant, IFN- $\gamma$ , IL-2, IL-12, monophosphoryl lipid A (MPL), Tre-  
 15 holose Dimycolate (TDM), Trehalose Dibehenate and muramyl dipeptide (MDP).

Preparation of vaccines which contain peptide sequences as active ingredients is generally well understood in the art, as exemplified by U.S. Patents 4,608,251; 4,601,903; 4,599,231 and 4,599,230, all incorporated herein by reference.

20

Other methods of achieving adjuvant effect for the vaccine include use of agents such as aluminum hydroxide or phosphate (alum), synthetic polymers of sugars (Carbopol), aggregation of the protein in the vaccine by heat treatment, aggregation by reactivating with pepsin treated (Fab) antibodies to albumin, mixture with bacterial cells such as *C. parvum*  
 25 or endotoxins or lipopolysaccharide components of gram-negative bacteria, emulsion in physiologically acceptable oil vehicles such as mannide mono-oleate (Aracel A) or emulsion with 20 percent solution of a perfluorocarbon (Fluosol-DA) used as a block substitute may also be employed. Other possibilities involve the use of immune modulating substances such as cytokines or synthetic IFN- $\gamma$  inducers such as poly I:C in combination with  
 30 the above-mentioned adjuvants.

Another interesting possibility for achieving adjuvant effect is to employ the technique described in Gosselin *et al.*, 1992 (which is hereby incorporated by reference herein). In brief, a relevant antigen such as an antigen of the present invention can be conjugated to



an antibody (or antigen binding antibody fragment) against the Fc $\gamma$  receptors on monocytes/macrophages.

- The vaccines are administered in a manner compatible with the dosage formulation, and
- 5 in such amount as will be therapeutically effective and immunogenic. The quantity to be administered depends on the subject to be treated, including, e.g., the capacity of the individual's immune system to mount an immune response, and the degree of protection desired. Suitable dosage ranges are of the order of several hundred micrograms active ingredient per vaccination with a preferred range from about 0.1  $\mu$ g to 1000  $\mu$ g, such as in
- 10 the range from about 1  $\mu$ g to 300  $\mu$ g, and especially in the range from about 10  $\mu$ g to 50  $\mu$ g. Suitable regimens for initial administration and booster shots are also variable but are typified by an initial administration followed by subsequent inoculations or other administrations.
- 15 The manner of application may be varied widely. Any of the conventional methods for administration of a vaccine are applicable. These are believed to include oral application on a solid physiologically acceptable base or in a physiologically acceptable dispersion, parenterally, by injection or the like. The dosage of the vaccine will depend on the route of administration and will vary according to the age of the person to be vaccinated and, to a
- 20 lesser degree, the size of the person to be vaccinated.

- The vaccines are conventionally administered parenterally, by injection, for example, either subcutaneously or intramuscularly. Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral for-
- 25 mulations. For suppositories, traditional binders and carriers may include, for example, polyalkalene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1-2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose,
- 30 magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and advantageously contain 10-95% of active ingredient, preferably 25-70%.

- In many instances, it will be necessary to have multiple administrations of the vaccine.
- 35 Especially, vaccines can be administered to prevent an infection with virulent mycobacte-

ria and/or to treat established mycobacterial infection. When administered to prevent an infection, the vaccine is given prophylactically, before definitive clinical signs or symptoms of an infection are present. Since the current vaccine BCG appears to induce an effective, but short-lived immune response, prophylactic vaccines may also be designed to be used as  
 5 booster vaccines. Such vaccines are given to individuals who have previously received a vaccination, with the intention of prolonging the period of protection. In instances where the individual has already become infected or is suspected to have become infected, the previous vaccination may have provided sufficient immunity to prevent primary disease, but as discussed previously, boosting this immune response will not help against the la-  
 10 tent infection. In such a situation, the vaccine will necessarily have to be a therapeutic vaccine designed for efficacy against the latent stage of infection.

Due to genetic variation, different individuals may react with immune responses of varying strength to the same polypeptide. Therefore, the vaccine according to the invention may  
 15 comprise several different polypeptides in order to increase the immune response. The vaccine may comprise two or more polypeptides or immunogenic portions, where all of the polypeptides are as defined above, or some but not all of the peptides may be derived from virulent mycobacteria. In the latter example, the polypeptides not necessarily fulfilling the criteria set forth above for polypeptides may either act due to their own immunogenic-  
 20 ity or merely act as adjuvants.

The vaccine may comprise 1-20, such as 2-20 or even 3-20 different polypeptides or fusion polypeptides, such as 3-10 different polypeptides or fusion polypeptides.

25 The invention also pertains to a method for immunising an animal, including a human being, against TB caused by virulent mycobacteria, comprising administering to the animal the polypeptide of the invention, or a vaccine composition of the invention as described above, or a living vaccine described above.

30 The invention also pertains to a method for producing an immunologic composition according to the invention, the method comprising preparing, synthesising or isolating a polypeptide according to the invention, and solubilizing or dispersing the polypeptide in a medium for a vaccine, and optionally adding other *M. tuberculosis* antigens and/or a carrier, vehicle and/or adjuvant substance.

### *DNA Vaccine.*

The nucleic acid fragments of the invention may be used for effecting *in vivo* expression of antigens, *i.e.* the nucleic acid fragments may be used in so-called DNA vaccines as reviewed in Ulmer et al 1993, which is included by reference.

5

Hence, the invention also relates to a vaccine comprising a nucleic acid fragment according to the invention, the vaccine effecting *in vivo* expression of antigen by an animal, including a human being, to whom the vaccine has been administered, the amount of expressed antigen being effective to confer substantially increased resistance to infections

10 caused by virulent mycobacteria in an animal, including a human being.

The efficacy of such a DNA vaccine can possibly be enhanced by administering the gene encoding the expression product together with a DNA fragment encoding a polypeptide which has the capability of modulating an immune response.

15

### *Live recombinant vaccines*

One possibility for effectively activating a cellular immune response for a vaccine can be achieved by expressing the relevant antigen in a vaccine in a non-pathogenic microorganism or virus. Well-known examples of such microorganisms are *Mycobacterium bovis*  
 20 BCG, *Salmonella* and *Pseudomona* and examples of viruses are Vaccinia Virus and Adenovirus.

Therefore, another important aspect of the present invention is an improvement of the living BCG vaccine presently available, wherein one or more copies of a DNA sequence encoding one or more polypeptide as defined above has been incorporated into the genome  
 25 of the micro-organism in a manner allowing the micro-organism to express and secrete the polypeptide. The incorporation of more than one copy of a nucleotide sequence of the invention is contemplated to enhance the immune response.

30 Another possibility is to integrate the DNA encoding the polypeptide according to the invention in an attenuated virus such as the vaccinia virus or Adenovirus (Rolph et al 1997). The recombinant vaccinia virus is able to replicate within the cytoplasm of the infected host cell and the polypeptide of interest can therefore induce an immune response, which is envisioned to induce protection against TB.

*Therapeutic vaccine.*

The invention also relates to the use of a polypeptide or nucleic acid of the invention for use as therapeutic vaccines as have been described by D. Lowrie (Lowrie, 1999) using DNA vaccine encoding HSP65 from *M. leprae*. Antigens with therapeutic properties may  
 5 be identified based on their ability to diminish the severity of *M. tuberculosis* infection in experimental animals or prevent reactivation of previous infection, when administered as a vaccine. The composition used for therapeutic vaccines can be prepared as described above for vaccines.

*Diagnostic protein*

10 The invention also relates to a method of diagnosing latent TB caused by a virulent mycobacterium in an animal, including a human being, comprising intradermally injecting, in the animal, a polypeptide according to the invention, a positive skin response at the location of injection being indicative of the animal having TB, and a negative skin response at the location of injection being indicative of the animal not having TB.

15

When diagnosis of latent infection with virulent mycobacteria is the aim, a blood sample comprising mononuclear cells (*i.e.* T-lymphocytes) from a patient is contacted with a sample of one or more polypeptides of the invention. This contacting can be performed *in vitro* and a positive reaction could e.g. be proliferation of the T-cells or release of cytokines

20 such as IFN- $\gamma$  into the extracellular phase. It is also conceivable to contact a serum sample from a subject with a polypeptide of the invention, the demonstration of a binding between antibodies in the serum sample and the polypeptide being indicative of previous or ongoing infection.

25 The invention therefore also relates to an *in vitro* method for diagnosing latent infection in an animal or a human being with a virulent mycobacterium, the method comprising providing a blood sample from the animal or human being, and contacting the sample from the animal with the polypeptide of the invention, a significant release into the extracellular phase of at least one cytokine by mononuclear cells in the blood sample being indicative  
 30 of the animal being sensitised. A positive response being a response more than release from a blood sample derived from a patient without the TB diagnosis plus two standard deviations. The invention also relates to the *in vitro* method for diagnosing ongoing or previous sensitisation in an animal or a human being with a virulent mycobacterium, the method comprising providing a blood sample from the animal or human being, and by  
 35 contacting the sample from the animal with the polypeptide of the invention demonstrating

the presence of antibodies recognizing the polypeptide of the invention in the serum sample.

The immunogenic composition used for diagnosing may comprise 1-20, such as 2-20 or 5 even 3-20 different polypeptides or fusion polypeptides, such as 3-10 different polypeptides or fusion polypeptides.

#### *Diagnostic DNA*

The nucleic acid probes encoding the polypeptide of the invention can be used in a variety of diagnostic assays for detecting the presence of pathogenic organisms in a given sample. 10

A method of determining the presence of mycobacterial nucleic acids in an animal, including a human being, or in a sample, comprising administering a nucleic acid fragment of the invention to the animal or incubating the sample with the nucleic acid fragment of the invention or a nucleic acid fragment complementary thereto, and detecting the presence 15 of hybridised nucleic acids resulting from the incubation (by using the hybridisation assays which are well-known in the art), is also included in the invention. Such a method of diagnosing TB might involve the use of a composition comprising at least a part of a nucleotide sequence as defined above and detecting the presence of nucleotide sequences in a sample from the animal or human being to be tested which hybridise with the nucleic acid 20 fragment (or a complementary fragment) by the use of PCR technique.

#### *Antibodies*

A monoclonal or polyclonal antibody, which is specifically reacting with a polypeptide of the invention in an immunoassay, or a specific binding fragment of said antibody, is also a part of the invention. The antibodies can be produced by methods known to a person 25 skilled in the art. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of a polypeptide according to the present invention and, if desired, an adjuvant. The monoclonal antibodies according to the present invention may, for example, be produced by the hybridoma method first described by Kohler and Milstein (Kohler and Milstein, 1975), or may be produced by recombinant DNA methods such as described in 30 U.S. Pat. No. 4,816,567. The monoclonal antibodies may also be isolated from phage libraries generated using the techniques described by McCafferty et al (McCafferty, 1990), for example. Methods for producing antibodies are described in the literature, e.g. in US6136958.

- A sample of a potentially infected organ or body fluid from an infected individual may be contacted with such an antibody recognizing a polypeptide of the invention. The demonstration of the reaction by means of methods well known in the art between the sample and the antibody will be indicative of an ongoing infection. It is of course also a possibility to demonstrate the presence of anti-mycobacterial antibodies in serum or other body fluids by contacting a serum sample from a subject with at least one of the polypeptide fragments of the invention and using well-known methods for visualising the reaction between the antibody and antigen.
- 10 In diagnostics, an antibody, a nucleic acid fragment and/or a polypeptide of the invention can be used either alone, or as a constituent in a composition. Such compositions are known in the art, and comprise compositions in which the antibody, the nucleic acid fragment or the polypeptide of the invention is coupled, preferably covalently, to at least one other molecule, e.g. a label (e.g. radioactive or fluorescent) or a carrier molecule.
- 15

## Figure Legends

### Figure 1. TB vaccination models.

- 20 A schematic time schedule of the models for A) prophylactic vaccination and B) therapeutic vaccination. Each square on the time axis represents one week. Three prophylactic vaccinations two weeks apart are given 6 weeks prior to an aerosol infection. The protective effect of the vaccines is measured 6 weeks after infection, in the acute phase of the infection. For analysis of therapeutic vaccinations a reactivation model is established,
- 25 where aerosol infected mice are treated with anti-M tuberculosis drugs for 8 weeks from the peak of infection (6 weeks after infection). This induces a latent infection phase with a low bacterial load. Four –five weeks into the latency phase three therapeutic vaccinations are given two weeks apart and the protective effect of the vaccines is measured as bacterial load in the reactivation phase, seven weeks after the last immunization.

30

### Fig 2. Prophylactic and therapeutic vaccine induced protection.

C57Bl/6j mice were immunized 3 times with a 2-week interval with recombinant ESAT6, BCG or recombinant Rv2031c. In A) the immunization was given as a prophylactic vaccine 6 weeks before the mice were given a *M. tuberculosis* infection (approx. 250 bacilli)

through the aerosol route with. Bacterial numbers in the lung was enumerated 6 weeks post infection. In B) the immunization was given as a therapeutic vaccine after a latent infection had been established. Bacterial numbers in the lung was enumerated 8 weeks after the last immunization. The data represents the mean of 5 individual mice.

5

**Fig 3. Rv2031c specific IFN- $\gamma$  responses.**

Latent infected C57Bl/6j mice were either not immunized or immunized with 3 $\mu$ g recombinant Rv2031 3 times with a two-week interval. One and two weeks post immunization mice were bleed and PBMCs isolated. The frequency of IFN- $\gamma$  producing cells specific for either ESAT6 or Rv2031c was determined for both the rRv2031c immunized and the unimmunized group. In an ELISPOT plate precoated with anti-IFN- $\gamma$  antibodies graded numbers of PBMCs were incubated with either 2 $\mu$ g/ml rRv2031c or 2 $\mu$ g/ml rESAT6. After 32h the plate was washed and incubated with biotinylated anti-IFN- $\gamma$  antibodies followed by a streptavidin-alkalinephosphatase incubation. The INFg spots, representing individual IFN- $\gamma$  producing cells were visualized using BCIP/NBT substrate. The results are shown as number Rv2031c specific IFN- $\gamma$  producing cell (black bars) and number of ESAT6 specific IFN- $\gamma$  producing cell (hatch bars) per 10<sup>6</sup> PBMCs.

**Fig 4. Epitope screening of Rv2031c.**

PBMCs from rRv2031c immunized latently infected C57Bl/6j mice were analyzed for recognition of 20'mer overlapping peptides scanning through Rv2031c. In A) the peptides were analyzed in pools of 3-4 peptides. PBMCs (2x10<sup>5</sup>) were incubated for 72 h with the peptide pools at 5  $\mu$ g/ml per peptide. Supernatant was harvested and secreted IFN- $\gamma$  was quantitated by ELISA. In B) individual peptides of positive pools were reanalyzed. PBMCs (2x10<sup>5</sup>) were incubated for 72 h with 1  $\mu$ g/ml of each peptide. Secreted IFN- $\gamma$  in the supernatant was quantitated.

**Fig 5. Protection against reactivation conferred by therapeutic vaccine given during latent infection.**

Latent infected C57Bl/6j mice were immunized 3 times with or without rRv2031c. Bacterial numbers in lung (A) and spleen (B) was enumerated 8 weeks after the last immunization. The data represents the mean of 8 individual mice.

35

## Examples

### **EXAMPLE 1: Cloning and expression of low oxygen induced *M. tuberculosis* antigens in *E. coli*.**

- 5 A number of *M. tuberculosis* genes are induced under low oxygen conditions. The up-regulation of the genes listed in table 2 has been determined at either the mRNA (Sherman, 2001) or protein (Boon, 2001, Rosenkrands, 2002) level. The coding region of these selected antigens is amplified by PCR using the primer sets listed in table 3.





**Table 3. Primer sequences for PCR amplification of selected low oxygen induced antigens**

Rv no.	Primer sequence	Ref
Rv0079	Fwd CACCGTGGAAACCGAAACGCAGTCG	[1]
	Rvs TTATGCCAGACCGTCGGCA	
Rv0080	Fwd CACCATGAGCCCGGGCTCG	[1]
	Rvs TTACGGCGTACGCGAGTCAG	
Rv0081	Fwd CACCGTGGAGTCCGAACCGCTGTA	[1]
	Rvs TTACGTGGCCGAGCCGC	
Rv0363c ( <i>fba</i> )	Fwd CACCATGCCTATCGCAACGCCC	[2]
	Rvs TTAGTGGGTTAGGGACTTTCCGG	
Rv0569	Fwd GGGGACAAGTTTGTACAAAAAGCAGGCTTAAAGG- CAAAGGTCGGGGAC	[1, 2]
	Rvs GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAC- GTTCCCTGGCATGGA	
Rv0572c	Fwd CACCATGGGTGAGCACGCCATC	[1]
	Rvs TTATAGGTCATCGGATTGAGGTGATC	
Rv0574c	Fwd CACCGTGGCTGGCAATCCTGATGT	[1]
	Rvs TTACTCCTTGCTCGTTAGGTTGGC	
Rv1264	Fwd CACCGTGACAGACCAGTGC GC	[1]
	Rvs TTACGGTGACGAGCCGGC	
Rv1592c	Fwd CACCATGGTAGAGCCCGGCAATTTG	[1]
	Rvs TTAGAGCGGACGGCGGCT	
Rv1733c	Fwd CACCATGATCGCCACAACCCGC	[1]
	Rvs TTACCGCTGCGTGCAGAACA	
Rv1734c	Fwd CACCATGACCAACGTCGGTGACCA	[1]
	Rvs TTATCCTGTTACTGCGGCGCA	
Rv1736c ( <i>narX</i> )	Fwd CACCGTGACGGTGACACCACGGAC	[1]
	Rvs TTACCACCCGCGCCGC	
Rv1737c ( <i>narK2</i> )	Fwd CACCATGAGAGGGCAAGCGGC	[1]
	Rvs TTACCTGGACGCCTCCTCACTC	
Rv1738	Fwd CACCATGTGCGGCGACCAGTC	[1]
	Rvs TTAATACAACAATCGCGCCGG	
Rv1739c	Fwd CACCATGATTCCACGATGACATCG	[1]
	Rvs TTAGCGCCGACGGAACG	
Rv1813c	Fwd GGGGACAAGTTTGTACAAAAAGCAGGCTTAATCA- CAAACCTCCGACGC	[1]
	Rvs GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAGTTGCAC- GCCCAGTTGAC	
Rv1997 ( <i>ctpF</i> )	Fwd CACCTTGTCGGCGTCAGTGTCTGC	[1]
	Rvs TTATGGCGGTTGCGCCC	
Rv1998c	Fwd CACCATGAGTTTCCACGATCTTCATCACC	[1]
	Rvs TTACGTTGTACTCGTGC GGTTCTC	
Rv2003c	Fwd CACCGTGGTCAAGCGCTCTCGG	[1]
	Rvs TTATTCCGACTCGAGTGGGTGA	

**Table 3. (continued) Primer sequences for PCR amplification of selected low oxygen induced antigens**

Rv no.	Primer sequence	Ref
Rv2005c	Fwd CACCATGTCTAAACCCCGCAAGCA	[1]
	Rvs TTACGACTGCCGTGCCACG	
Rv2007c ( <i>fdxA</i> )	Fwd CACCGTGACCTATGTGATCGGTAGTGAGTG	[1]
	Rvs TTAAGGGCACTCCACCGGGA	
Rv2028c	Fwd CACCATGAACCAATCACACAAACCCC	[1]
	Rvs TTACAGATACTGCTGACCGACGACC	
Rv2029c ( <i>pfkB</i> )	Fwd CACCATGACGGAGCCAGCGG	[1]
	Rvs TTATGGCGAGGCTTCCGG	
Rv2030c	Fwd GGGGACAAGTTTGTACAAAAAGCAGGCTTACTGAT- GACCGCAGCGGCT	[1]
	Rvs GGGGACCACTTTGTACAAGAAAGCTGGGTCCTACA- GACCGGTCGGGTAGGTTT	
Rv2031c ( <i>hspX</i> )	Fwd GGGGACAAGTTTGTACAAAAAGCAGGCTTAGCCAC- CACCTTCCCGT	[1-3]
	Rvs GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAGTT- GGTGGACCGGATCTGAAT	
Rv2032	Fwd CACCATGCCGGACACCATGGTG	[1]
	Rvs TTAGTGATCCTTAGCCCGAACGTG	
Rv2428 ( <i>ahpC</i> )	Fwd GGGGACAAGTTTGTACAAAAAGCAGGCTTAATGCCACT- GCTAACCATTGGC	[1]
	Rvs GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAGGCC- GAAGCCTTGAGGAGT	
Rv2623	Fwd CTGAGATCTATGTCATCGGGCAATTCA	[1-3]
	Rvs CTCCCATGGCTACCTAAGTCAGCGACTCGCG	
Rv2624c	Fwd CACCATGTCTGGGAGAGGAGAGCCG	[1]
	Rvs TTAGCGAACGACAAGCACCGA	
Rv2625c	Fwd GGGGACAAGTTTGTACAAAAAGCAGGCTTACGTGAT- GCGATCCCGCT	[1]
	Rvs GGGGACCACTTTGTACAAGAAAGCTGGGTCCTACCCC- GCATCGGAAAACC	
Rv2626c	Fwd GGGGACAAGTTTGTACAAAAAGCAGGCTTAACCACC- GCACGCGACA	[1-3]
	Rvs GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAGCT- GGCGAGGGCCAT	
Rv2627c	Fwd GGGGACAAGTTTGTACAAAAAGCAGGCTTAAT- GGCAAGTTCTGCGAGCGA	[1]
	Rvs GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAG- GAACGGTCGCGCTGTGT	
Rv2628	Fwd CACCATGTCCACGCAACGACCG	[1]
	Rvs TTAACCGCAACGGCAATCTCA	

**Table 3. (continued) Primer sequences for PCR amplification of selected low oxygen induced antigens**

Rv no.	Primer sequence	Ref.
Rv2629	Fwd CACCATGCGATCAGAACGTCTCCG	[1]
	Rvs TTAGGATCTATGGCTGCCGAGTC	
Rv2630	Fwd CACCATGCTGCACCGCGACGA	[1]
	Rvs TTACACATCGAGCGTTACCGCAC	
Rv2659c	Fwd GGGGACAAGTTTGTACAAAAAGCAGGCTTAGTGAC-GCAAACCGGCAA	[1]
	Rvs GGGGACCACTTTGTACAAGAAAGCTGGGTCCTA-CATCTCCTGGTTCTCGGCC	
Rv2780	Fwd GGGGACAAGTTTGTACAAAAAGCAGGCTTAC-GCGTCGGTATTCCGACC	[2]
	Rvs GGGGACCACTTTGTACAAGAAAGCTGGGTCCTA-CACGCTGGCGGGCTC	
Rv3126c	Fwd CACCATGGTCATCCGGTTTGATCAAATA	[1]
	Rvs TTATGGATTGTCAATGACAGCCCA	
Rv3127	Fwd CACCGTGCTCAAGAACGCAGTCTTGC	[1]
	Rvs TTAAGGCGGGCTGAACCAACC	
Rv3128c	Fwd CACCGTGTGGTCCGCTCGG	[1]
	Rvs TTAGCCCGCCTTGATCAGGA	
Rv3129	Fwd CACCGTGGTGCAAGGCCGCA	[1]
	Rvs TTATCGCTGGTTGTGTGACGAG	
Rv3130c	Fwd CACCATGAATCACCTAACGACACTTGACG	[1]
	Rvs TTACACAACCAGCGATAGCGCTC	
Rv3131	Fwd CACCATGAACACCCATTTCCCGG	[1]
	Rvs TTAGCACCGTTGTGCGAGTAGCT	
Rv3132c	Fwd CACCATGACAACAGGGGGCCTCG	[1]
	Rvs TTAGTGCACAAACGGTGCTGAC	
Rv3133c	Fwd CACCGTGGTAAAGGTCTTCTTGGTCGAT	[1, 3]
	Rvs TTATGGTCCATCACCGGGTG	
Rv3134c	Fwd CACCATGAGCGATCCTCGGCCA	[1]
	Rvs TTACAAGTTGGCACTGCGTACCG	
Rv3841 ( <i>bfrB</i> )	Fwd CCGGCTGAGATCTATGACAGAATACGAAGGGC	[1, 2]
	Rvs CCCCAGGGAAGTAGAGGCGGC	
Rv3842c ( <i>glpQ1</i> )	Fwd CACCATGACATGGGCCGACGAG	[1]
	Rvs TTAGCGAGTGGTCCCGTTTCG	
Rv3908	Fwd CACCGTGTCCGACGGCGAACA	[1]
	Rvs TTACGGCCCCGGGCC	

PCR reactions were carried out using Platinum Tag DNA Polymerase (GIBCO BRL) in a 50  $\mu$ L reaction volume containing 60 mM Tris-SO<sub>4</sub> (pH 8.9), 18 mM Ammonium Sulfate, 0.2 mM of each of the four nucleotides, 0.2  $\mu$ M of each primer and 10 ng of *M. tuberculosis* H37Rv chromosomal DNA. The reaction mixtures were initially heated to 95° C for 5 min., followed by 35 cycles of: 95° C for 45 sec, 60° C for 45 sec and 72° C for 2 min. The amplification products were precipitated by PEG/MgCl<sub>2</sub>, and dissolved in 50  $\mu$ L TE buffer.

DNA fragments were cloned and expressed in Gateway Cloning system (Life Technology). First, to create Entry Clones, 5  $\mu$ L of DNA fragment was mixed with 1  $\mu$ L of pDONR201, 2  $\mu$ L of BP CLONASE enzyme mix and 2  $\mu$ L of BP reaction buffer. The recombination reactions were carried out at 25° C for 60 min. After Proteinase K treatment at 37° C for 10 min., 5  $\mu$ L of each sample was used to transform *E. coli* DH5 $\alpha$  competent cells. Transformants were selected on LB plates containing 50  $\mu$ g/mL kanamycin. One bacterial clone from each transformation was grown in 3 mL LB medium containing 50  $\mu$ g/mL kanamycin and plasmid DNA was isolated (Qiagen).

Second, to create expression clones, 2  $\mu$ L of each entry clone DNA was mixed with 1  $\mu$ L of His-tagged expression vector (pDest17), 2  $\mu$ L LR reaction buffer, 2  $\mu$ L LR CLONASE enzyme mix and 3  $\mu$ L TE. After recombination at 25° C for 60 min. and Proteinase K treatment at 37° C for 10 min., 5  $\mu$ L of each sample was used to transform *E. coli* BL21-SI competent cells. Transformants were selected on LBON (LB without NaCl) plates containing 100  $\mu$ g/mL ampicillin. The resulting *E. coli* clones express recombinant proteins carrying a 6-histidine tag at the N-terminal. All clones were confirmed by DNA sequencing.

Recombinant proteins were purified from transformed *E. coli* BL21-SI cells cultured in 900 mL LBON medium containing 100  $\mu$ g/mL at 30° C until OD<sub>600</sub> = 0.4-0.6. At this point 100 mL 3 M NaCl was added and 3 hours later bacteria were harvested by centrifugation. Bacteria pellets were resuspended in 20 mL bacterial protein extraction reagent (Pierce) incubated for 10 min. at room temperature and pelleted by centrifugation. Bacteria were lysed and their DNA digested by treating with lysozyme (0.1 mg/mL) and DNase I (2.5  $\mu$ g/mL) at room temperature for 30 minutes, with gentle agitation. The recombinant protein forms inclusion bodies and can be pelleted by centrifugation at 27,000 x g for 15 min. Protein pellets were solubilized by adding 20 mL of sonication buffer (8 M urea, 50 mM Na<sub>2</sub>HPO<sub>4</sub>, 100 mM Tris-HCl, pH 8.0) and sonicating 5 x 30 sec pulses interrupted by a 30 sec pause. After another centrifugation at 27,000 x g for 15 min., supernatants were applied to 10 mL TALON columns (Clontech). The columns were washed with 50 mL sonication buffer. Bound proteins were eluted by lowering pH (8 M urea, 50 mM Na<sub>2</sub>HPO<sub>4</sub>, 100 mM Tris-HCl, pH 4.5). 5 mL fractions were collected and analyzed by SDS-PAGE. Fractions containing recombinant protein were pooled. Further purification was achieved by anion- or cation- exchange chromatography on Hitrap columns (Pharmacia). Bound protein was eluted using a NaCl gradient from 0 – 500 mM in 3 M urea, 10 mM Tris-HCl,

pH 8.0. All fractions were collected and analyzed on SDS-PAGE using Coomassie staining. Fractions containing recombinant protein were pooled. Final protein concentrations were determined by micro BCA (Pierce).

5

## **EXAMPLE 2: Prophylactic versus therapeutic vaccine.**

### **Murine vaccination models.**

- A prophylactic vaccine given prior to infection should induce an immune response sufficiently strong to prevent or dampen the initial proliferation of the bacteria in the acute phase and thereby reduce the ensuing disease. In the murine prophylactic vaccine model outlined in figure 1A, naïve mice are immunized 3 times, 2 weeks apart with recombinant antigens. Six weeks after the last immunization, the mice are given an aerosol infection with approximately 250 *M. tuberculosis* bacilli. The protective capacity of the vaccine is evaluated by enumeration of the bacteria in spleen and lung 6 weeks post-infection.
- To define the optimal components for a therapeutic vaccine, a murine reactivation model of latent TB has been established (van Pinxteren, 2000) (figure 1B). An aerosol infection with approximately 250 *M. tuberculosis* bacilli is given and at the peak of infection 6 weeks after, the mice receive an 8-week course of anti-mycobacterial drug treatment of isoniazid and rifabutin given in the drinking water. This reduces the bacterial load in spleen and lung to a low level (about 500 bacteria per organ). This latent phase of low chronic infection is stable for 9-10 weeks after which a slow spontaneous reactivation occurs. The therapeutic vaccine is given as 3 subcutaneous (s.c.) immunizations about 5 weeks after cessation of drug treatment. The effect of the therapeutic vaccine is evaluated as protection against reactivation determined by enumeration of bacteria in spleen and lung 7 weeks after the last immunization.

### **The effect of the antigens in a prophylactic or a therapeutic vaccine.**

- BCG, ESAT6, and Rv2031c, one of the most prominent proteins induced under low oxygen conditions (Rosenkrands, 2002), were analyzed for their prophylactic and therapeutic vaccine potential. Naïve or latently infected C57Bl mice were immunized with one s.c. injection of  $2.5 \times 10^5$  BCG, or 3 s.c. immunizations of 10 µg of either recombinant ESAT6 or recombinant Rv2031c in a DDA/MPL adjuvant. The vaccinations were done in groups of 5 mice and protective capacity of the vaccines was evaluated as described above. Figure 2 shows the bacterial load in the lung in the acute phase (A) and in the reactivation phase (B), after prophylactic and therapeutic vaccination respectively. ESAT6 (as previously described by Brandt, 2000) offers protection against acute phase infection at the level of BCG (figure 2A). However, neither of the two shows any protective effect against reactivation of the infection when given during latent infection (figure 2B). In contrast, Rv2031c, the low oxygen induced antigen, offers no protection against the acute phase of the infection when given as a prophylactic vaccine, but gives some protection against reactivation when given as a therapeutic vaccine. That is, some antigens, here exemplified by ESAT6, though potent as prophylactic vaccines have no effect as therapeutic vaccines. In

contrast, other antigens, here exemplified by Rv2031c, could be efficient therapeutic vaccines although they have no effect or only negligible effect as prophylactic vaccines.

**Example 3. Low oxygen induced antigens, Rv2031c, as therapeutic vaccines:**

- 5 There is a high variability in bacterial load intrinsic to the reactivation model in the latent and reactivation phase. The analysis of Rv2031c as a therapeutic vaccine was therefore repeated in groups of eight mice. As in the previous experiments the mice were given 3 s.c. immunizations of 10 $\mu$ g rRv2031c in DDA/MPL. The induced immune responses were analyzed one week post immunization. The mice were partially bled and the PBMC from
- 10 the blood purified and analyzed for Rv2031c- and ESAT6 specific recall responses. Using ELIspot technique, the frequency of Rv2031c-specific and ESAT6-specific IFN- $\gamma$ -producing cells were determined in both the rRv2031c immunized and the unimmunized group (figure 3). The rRv2031c immunization has increased the frequency of Rv2031c-specific IFN- $\gamma$  producing cells by a factor of 43 as compared to the unimmunized group.
- 15 In contrast, the frequency of ESAT6-specific IFN- $\gamma$  producing cells is significantly higher in the unimmunized group. ESAT6 is an antigen produced in high amounts by the actively-growing *M. tuberculosis* bacteria. The level of the ESAT6 specific immune response in infected mice could therefore be indicative the degree of actively-growing infection in the animals. Recent reports have in fact demonstrated such a correlation between the level
- 20 of ESAT6 response and degree of disease in both *M. tuberculosis*-infected humans and *M. bovis*-infected cattle (Doherty, 2002, Vordermeier, 2002). Therefore, the higher ESAT6 response in the unimmunized group of latently-infected mice could be indicative of a transition into the reactivation phase, where the bacteria are again beginning to multiply. To analyze the epitope recognition pattern of Rv2031c, fourteen overlapping peptides
- 25 (each 20 amino acids long) covering the whole Rv2031c protein were synthesized. Initially the peptides were analyzed in 4 pools of 3-4 peptides. PBMCs from rRv2031c immunized latently-infected mice were incubated with the peptide pools (5  $\mu$ g/ml per peptide) for 72 h. The specific peptide-induced IFN- $\gamma$  production was quantitated in the supernatant in a standard sandwich ELISA using paired anti-murine IFN- $\gamma$  antibodies (PharMingen) and
- 30 recombinant IFN- $\gamma$  (PharMingen) as standard. Both peptide pool 1-4 and 8-10 stimulated a significant IFN- $\gamma$  response (figure 4A). The individual peptides of these two pools were re-analyzed (figure 4B). This clearly shows that the response to Rv2031c contains a dominant epitope, peptide 2 (PRSLFPEFSELFAAFPSFAG), and a subdominant epitope, peptide 9 (RTEQKDFDGRSEFAYGSFVR).
- 35 The therapeutic effect of the rRv2031c immunizations was analyzed 7 weeks after the last immunization. Figure 5 shows the bacterial load in the lung (A) and the spleen (B) of both rRv2031c-immunized and unimmunized mice. There is a clear reduction in the level of bacteria in both organs in the rRv2031c-immunized group. That is, the induction of Rv2031c T cell responses can participate in keeping the latent infection in check.

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## Claims

1. Use of one or more polypeptides or fragments hereof, which polypeptides are expressed during the latent stage of the mycobacterial infection, and/or nucleic acids encoding these polypeptides, for a therapeutic vaccine against tuberculosis.
2. Use according to claim 1, where the polypeptides upregulated during the latent stage of the mycobacterial infection, comprises one or more amino acid sequences selected from
  - (a) SEQ ID NO. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48 and 49
  - (b) an immunogenic portion, e.g. a T-cell epitope, of any one of the sequences in (a); and /or
  - (b) an amino acid sequence analogue having at least 70% sequence identity to any one of the sequences in (a) or (b) and at the same time being immunogenic.
3. Use according to claim 2, where the immunogenic portions are selected from the group consisting of SEQ ID NO 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48 and 49.
4. Use according to claims 1-3, where the nucleic acid sequences are selected from SEQ ID NO. 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97 and 98.
5. A therapeutic vaccine against tuberculosis comprising one or more polypeptides or fragments hereof, which polypeptides are expressed during the latent stage of the mycobacterial infection, and/or nucleic acids encoding these polypeptides.
6. A therapeutic vaccine according to claim 5 where the polypeptides upregulated during the latent stage of the mycobacterial infection, which stage is characterized by low-oxygen

tension in the microenvironment of the mycobacteria, comprises one or more amino acid sequences selected from

- (a) SEQ ID NO 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48 and 49
- (b) an immunogenic portion, e.g. a T-cell epitope, of any one of the sequences in (a); and /or
- (d) an amino acid sequence analogue having at least 70% sequence identity to any one of the sequences in (a) or (b) and at the same time being immunogenic.

10

7. A therapeutic vaccine according to claim 6, where the immunogenic portions are selected from the group consisting of SEQ ID NO 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48 and 49.

15

8. A therapeutic vaccine according to claims 5-7, where the polypeptides or fragments hereof, which polypeptides are expressed during the latent stage of the mycobacterial infection, which stage is characterized by low-oxygen tension in the microenvironment of the mycobacteria, are fused to other antigens expressed by bacteria within the mycobacteria family.

20

9. A therapeutic vaccine according to claim 8 where the fusionpartners is selected from the group consisting of ESAT-6, ESAT-6-Ag85B, TB10.4, CFP10, RD1-ORF5, RD1-ORF2, Rv1036, MPB64, MPT64, Ag85A, Ag85B (MPT59), MPB59, Ag85C, 19kDa lipoprotein, MPT32.

25

10. A therapeutic vaccine according to claims 5-9, where the nucleic acid sequence are selected from SEQ ID NO. 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97 and 98.

30

10. 11. A multiphase vaccine comprising antigene components with therapeutic activity according to claim 5-10 combined with antigen components with prophylactic activity.

12. A multiphase vaccine according to claim 11 where the antigen components with prophylactic activity comprises ESAT-6, ESAT-6-Ag85B, TB10.4, CFP10, RD1-ORF5, RD1-ORF2, Rv1036, MPB64, MPT64; Ag85A, Ag85B (MPT59), MPB59, Ag85C, 19kDa lipoprotein or MPT32.

5

13. A vaccine according to claim 5-12, comprising as the effective component a non-pathogenic microorganism, wherein at least one copy of a DNA fragment comprising an antigen component with therapeutic activity according to claim 5-10 or/and an antigen component with prophylactic activity has been incorporated into the genome of the microorganism in a manner allowing the microorganism to express and optionally secrete the polypeptide.

14. A vaccine according to claim 13 where the non-pathogenic microorganism is selected among bacteria or virus.

15

15. A vaccine according to claim 5-12, where antigen components are recombinant polypeptides or synthetic peptides delivered in a delivery system such as an adjuvant.

16. A vaccine according to claims 5-12 in which the amino acid sequence is lipidated so as to allow a self-adjuvanting effect of the polypeptide.

17. A method for treating an animal, including a human being, with tuberculosis caused by virulent mycobacteria, e.g. by *Mycobacterium tuberculosis*, *Mycobacterium africanum* or *Mycobacterium bovis*, comprising administering to the animal the vaccine according to claims 5-16.

18. A method for immunising an animal, including a human being, against tuberculosis caused by virulent mycobacteria, e.g. by *Mycobacterium tuberculosis*, *Mycobacterium africanum* or *Mycobacterium bovis*, comprising administering to the animal the vaccine according to claim 11-16.

19. A method of diagnosing tuberculosis caused by virulent mycobacteria, e.g. by *Mycobacterium tuberculosis*, *Mycobacterium africanum* or *Mycobacterium bovis*, in an animal, including a human being, comprising intradermally injecting, in the animal, polypeptides or fragments hereof, which polypeptides are expressed during the latent stage of the myco-

bacterial infection, and/or nucleic acids encoding these polypeptides, a positive skin response at the location of injection being indicative of the animal having tuberculosis, and a negative skin response at the location of injection being indicative of the animal not having tuberculosis.

5

18. A method for diagnosing previous or ongoing infection with a virulent mycobacterium, said method comprising contacting a sample, e.g. a blood sample, comprising mononuclear cells (e.g. T-lymphocytes), with a polypeptides or fragments hereof, which polypeptides are expressed during the latent stage of the mycobacterial infection, which stage is
- 10 characterized by low-oxygen tension in the microenvironment of the mycobacteria, in order to detect a positive reaction, e.g. proliferation of the cells or release of cytokines such as IFN- $\gamma$ .

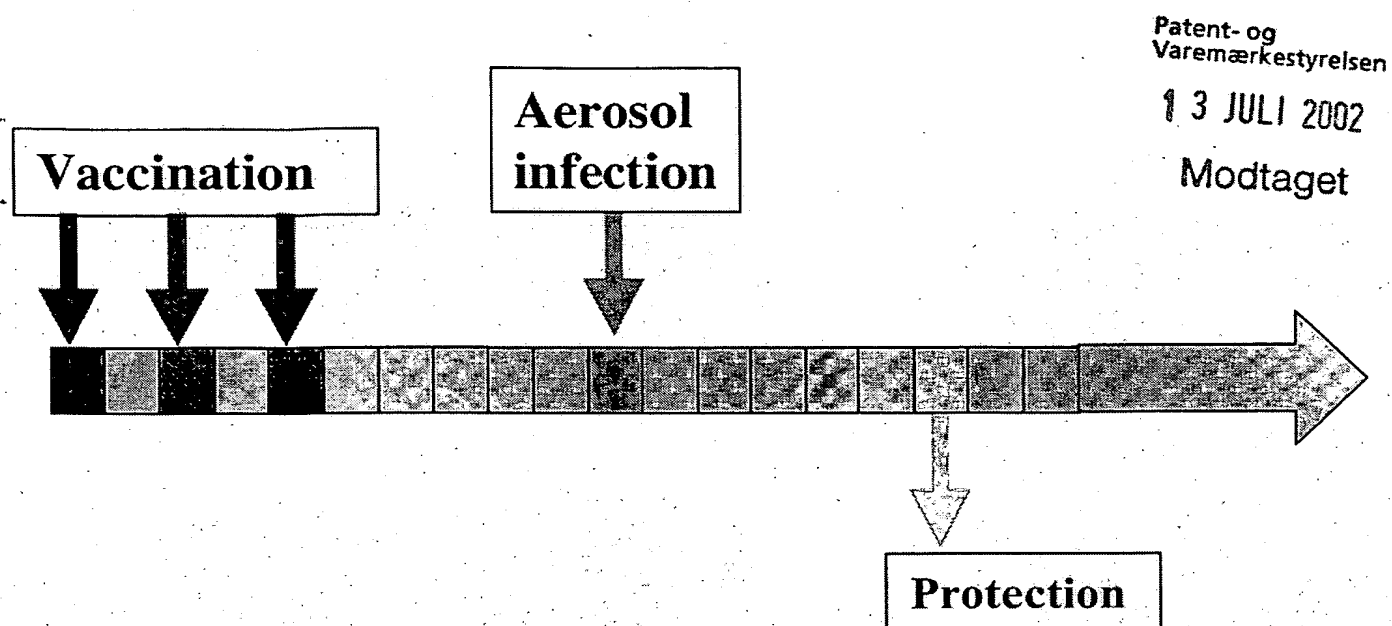
19. A method of diagnosing *Mycobacterium tuberculosis* infection in a subject comprising:

- 15 (a) contacting a polypeptides or fragments hereof, which polypeptides are expressed during the latent stage of the mycobacterial infection, which stage is characterized by low-oxygen tension in the microenvironment of the mycobacteria, with a bodily fluid of the subject;
- (b) detecting binding of an antibody to said polypeptide, said binding being an indication that said subject is infected by *Mycobacterium tuberculosis* or is susceptible
- 20 to *Mycobacterium tuberculosis* infection.

## **ABSTRACT**

The present invention is based on a number of *M. tuberculosis* derived proteins and protein fragments which are induced during the latent stage of infection characterized by low oxygen tension in the microenvironment of the infecting TB-bacteria. The invention is directed to the use of these polypeptides, immunologically active fragments thereof and the genes encoding them for immunological compositions such as therapeutic vaccines and diagnostic reagents.

## A) Infection model for prophylactic vaccination



## B) Latency model for therapeutic vaccination

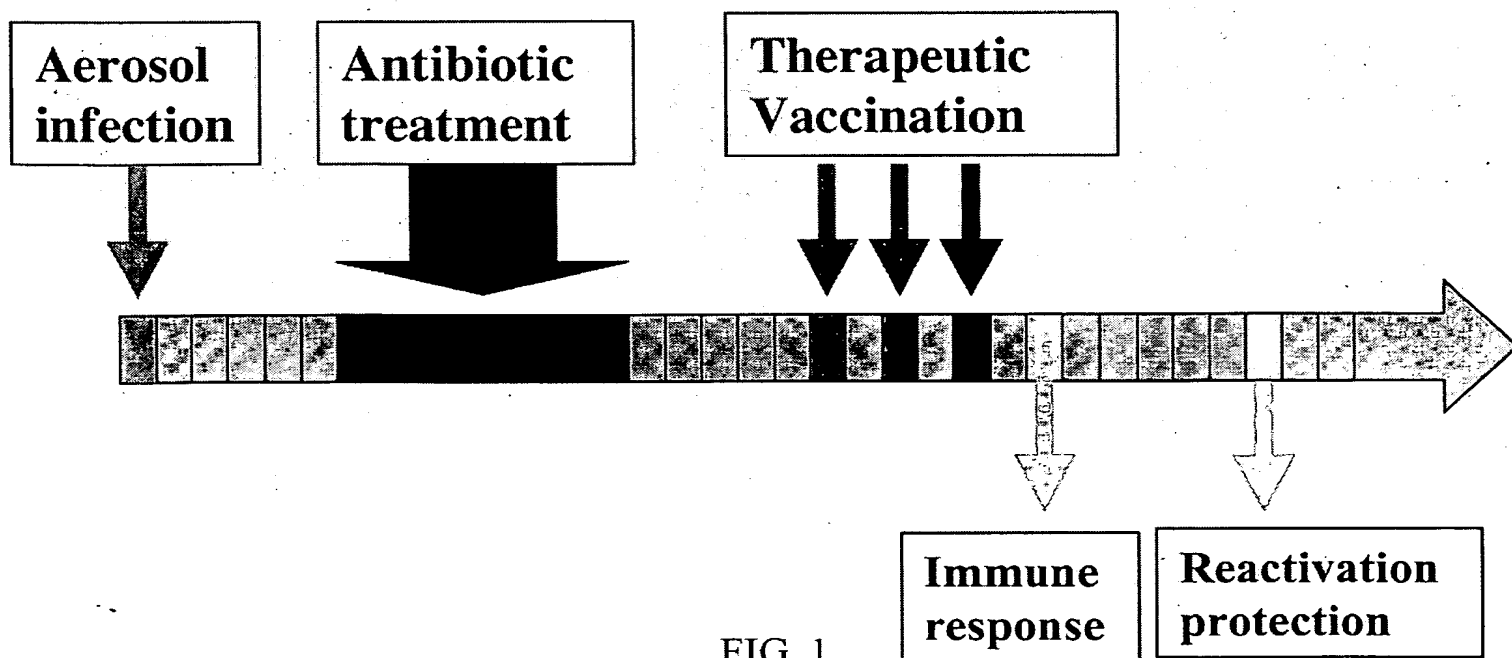


FIG. 1

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Modtaget

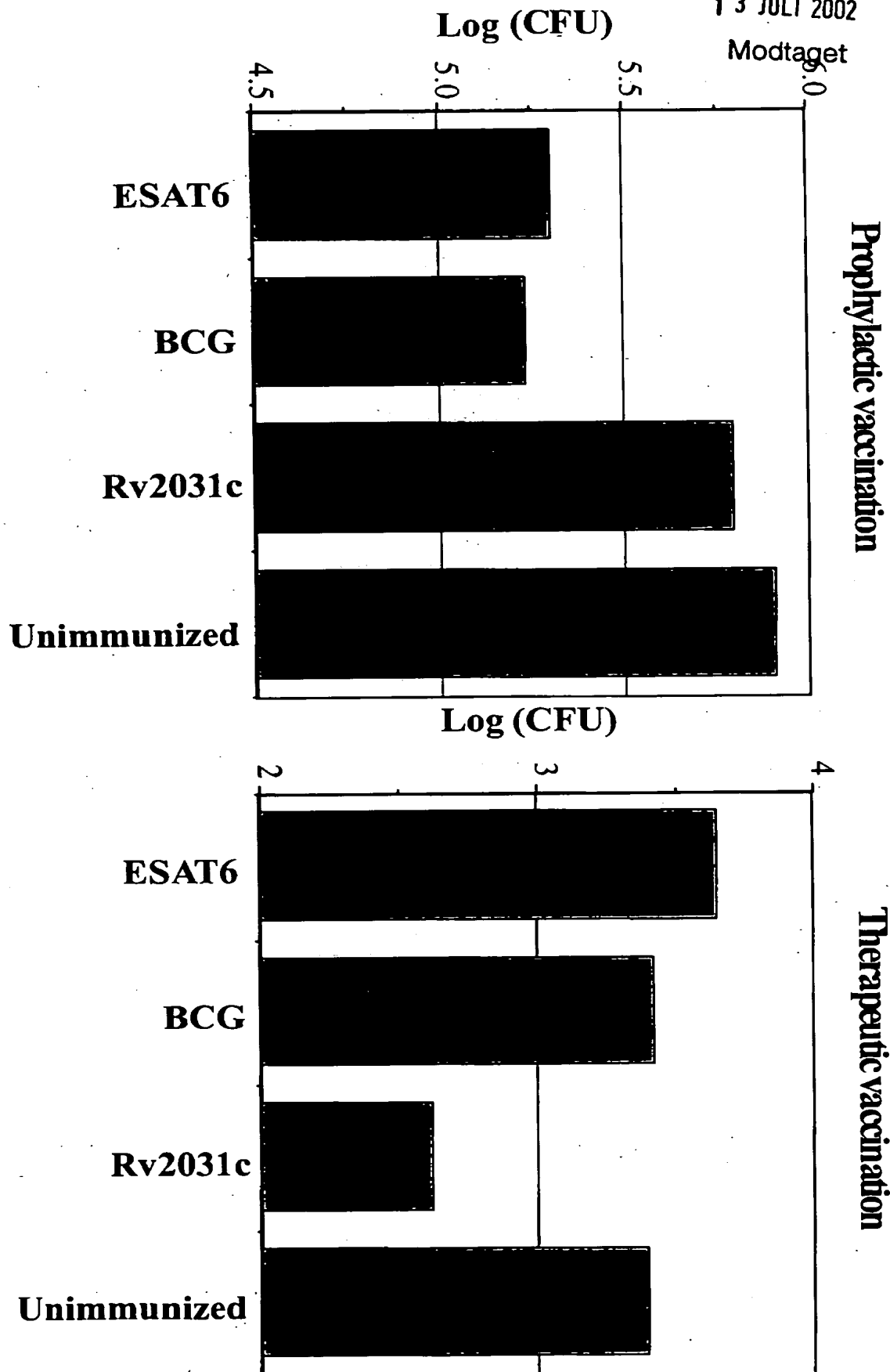


FIG. 2



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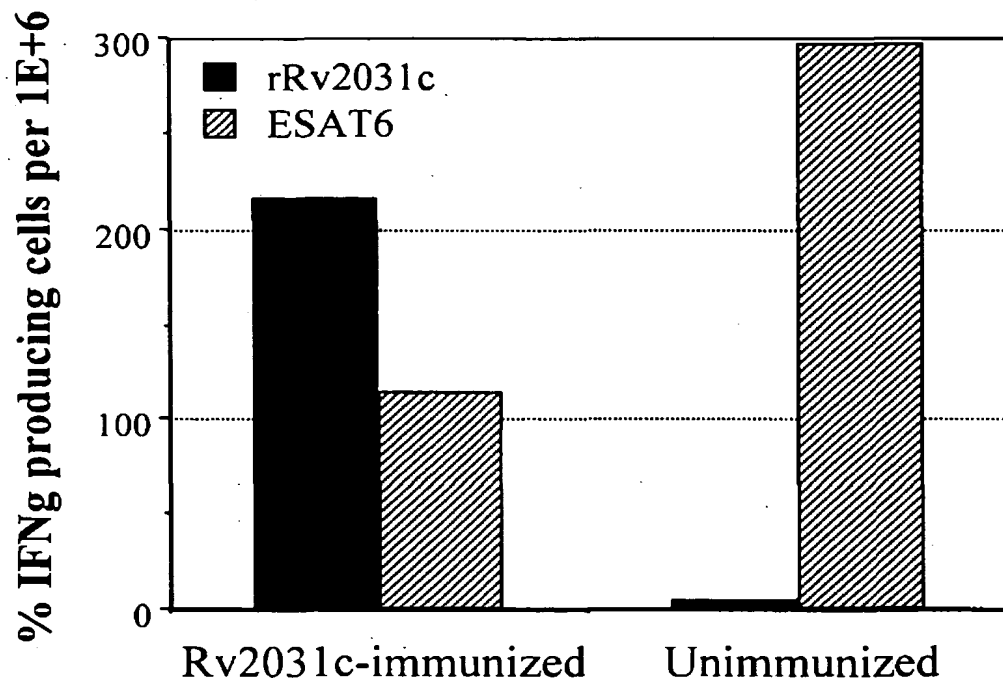


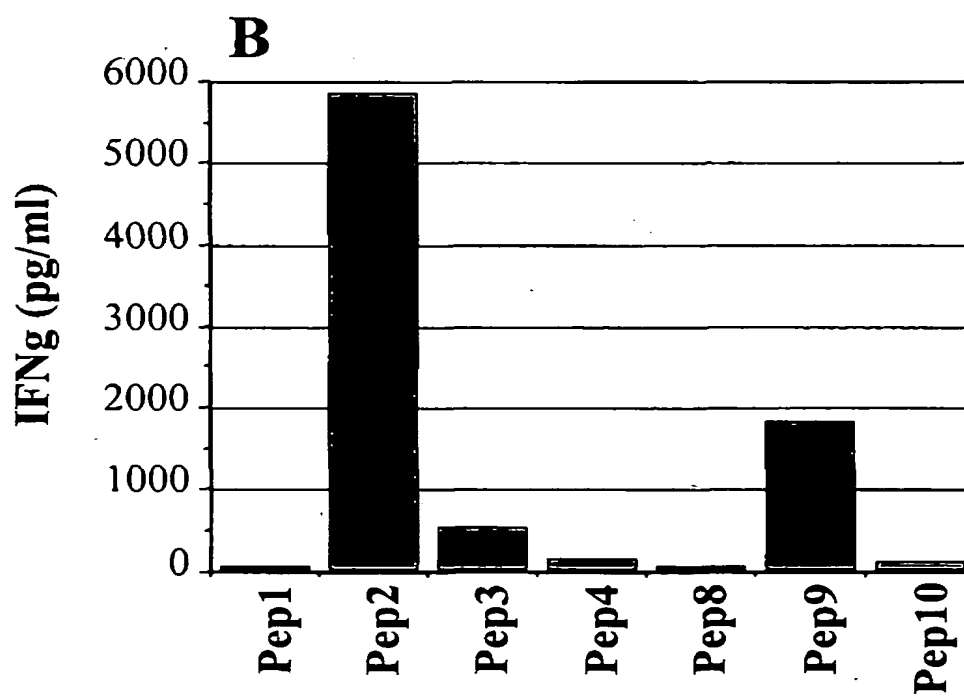
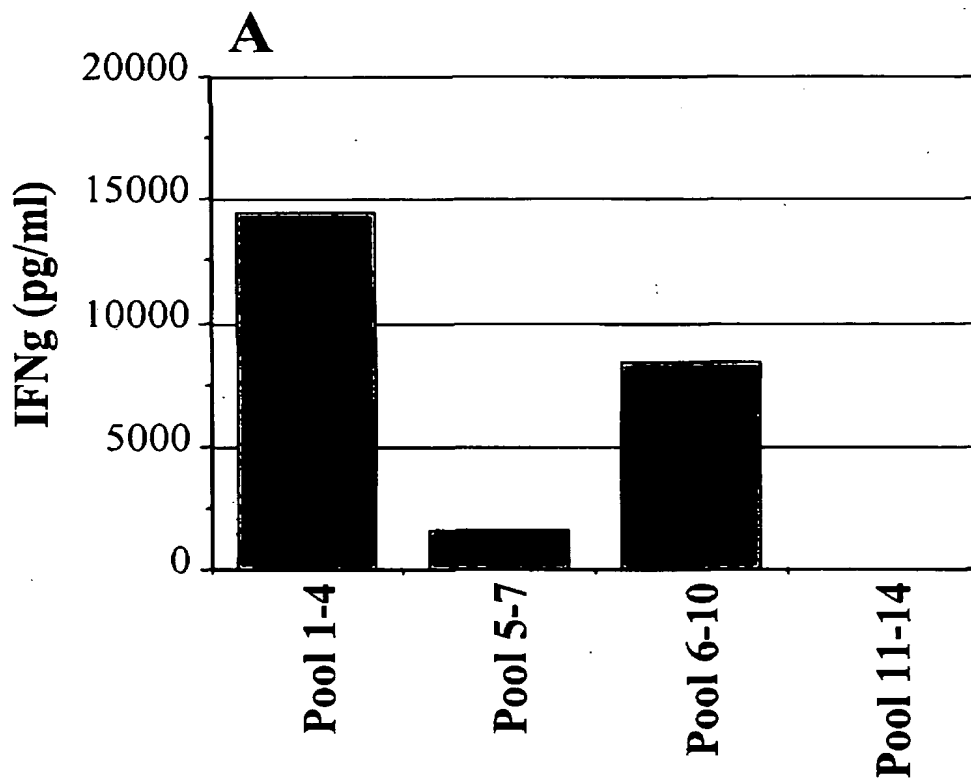
FIG. 3

FIG. 4

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Varemærkestyrelsen

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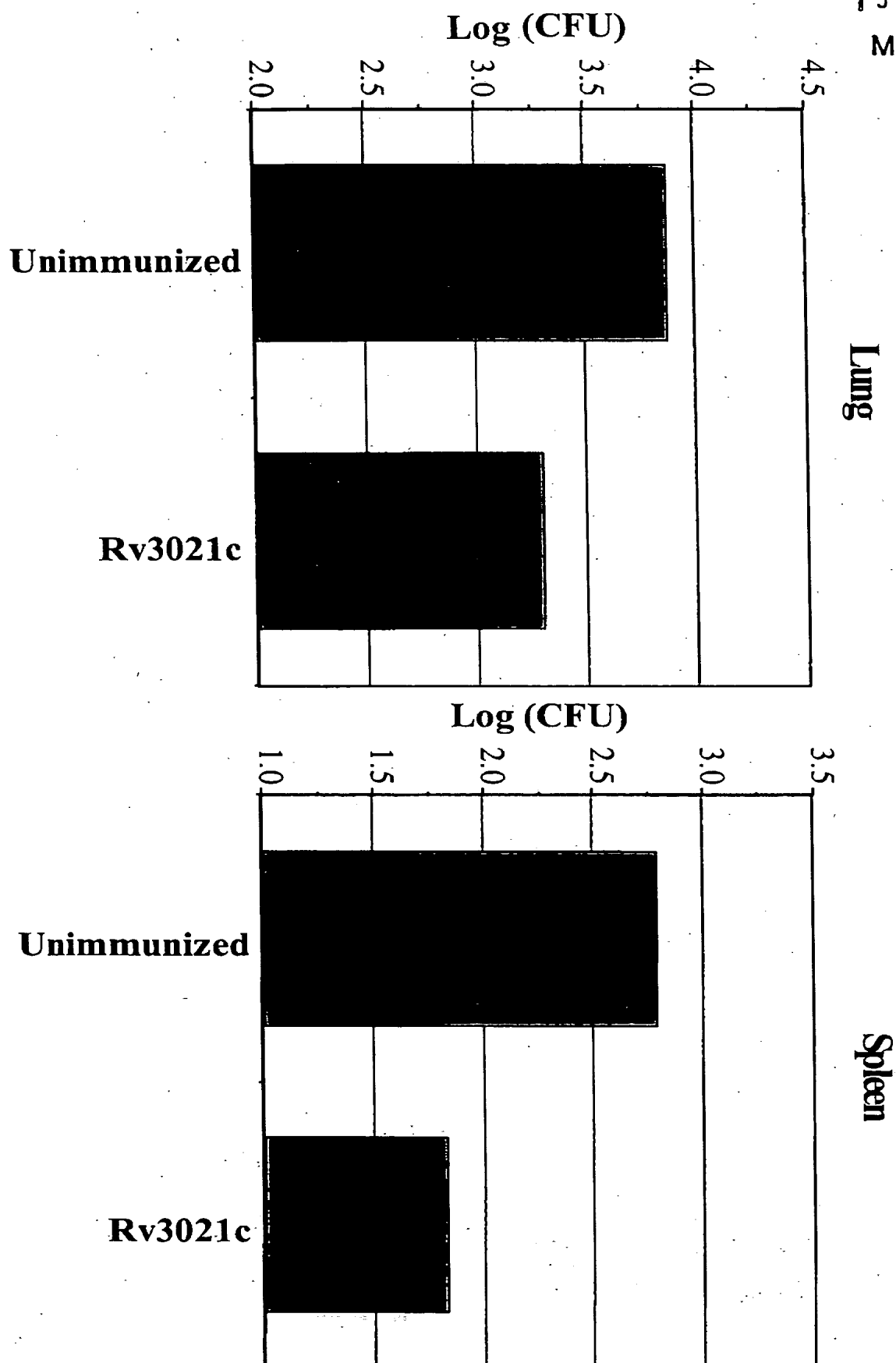


FIG. 5